Gene Drive-Modified Organisms: Developing Practical Risk Assessment Guidance

Yann Devos, Michael Bonsall, Leslie Firbank, John Mumford, Fabien Nogué, Ernst Wimmer

Title
Gene drive-modified organisms: developing practical risk assessment guidance

Authors
Yann Devos (https://orcid.org/0000-0002-4067-7871),[1,*]
Michael B Bonsall (https://orcid.org/0000-0003-0250-0423),[2]
Leslie G Firbank (https://orcid.org/0000-0003-1242-8293),[3]
John Mumford (https://orcid.org/0000-0001-8964-1784),[4]
Fabien Nogué (https://orcid.org/0000-0003-0619-4638),[5]
Ernst A Wimmer (https://orcid.org/0000-0002-2480-2804),[6]

Affiliations
[1] GMO Unit, European Food Safety Authority (EFSA), Parma, Italy
[2] Department of Zoology, University of Oxford, Oxford, United Kingdom
[3] School of Biology, University of Leeds, Leeds, United Kingdom
[4] Centre for Environmental Policy, Imperial College London, Ascot, United Kingdom
[5] Institut Jean-Pierre Bourgin, INRAE, AgroParisTech, Université Paris-Saclay, Versailles, France
[6] Johann Friedrich Blumenbach Institute of Zoology and Anthropology, GZMB, Georg August University, Göttingen, Germany

[*] Correspondence: yann.devos@efsa.europa.eu (Y. Devos)

Keywords
Engineered gene drives, modelling, monitoring, precautionary principle, problem formulation, synthetic biology

Abstract
Risk assessors, risk managers, developers, potential applicants and other stakeholders at many levels discuss the need for new or further risk assessment guidance for deliberate environmental releases of gene drive-modified organisms. However, preparing useful and practical guidance entails challenges, to which we offer recommendations based on our experience drafting guidance.

Engineered gene drives

Gene drives (GDs) are genetic elements capable of biasing their own inheritance [1]. The idea of harnessing naturally occurring GDs against disease vectors, agricultural pests and invasive species is not new. However, it proved difficult to engineer efficient GD systems using classical genetic approaches. Recent advances in molecular and synthetic biology enable practical GDs in a range of organisms, with most initial focus on insects. Use of engineered GDs is also proposed to complement efforts on biodiversity conservation.

In insects, GDs can be engineered to suppress wild target populations, or modify their genetic makeup into (e.g.) being less able to transmit disease or more resistant to infection (in disease vectors). Depending on the GD system, theoretically, a genetic modification could spread through target populations (non-localised) and persist indefinitely (self-sustaining), or be restricted in spread (localised) or persistence (self-limiting) [Table 1]. Inherent to many GDs is the need for initial releases above a threshold density before they will drive the genetic modification through target populations. Low threshold GDs may spread from very low initial release densities, with high potential to spread into neighbouring populations. High threshold GDs would only spread if the density of modified individuals reaches a high proportion within the target population, enabling local confinement [2-3].

Gene drive-modified insects (GDMIs) may address long-standing challenges in the control of disease vectors, pests and invasive species [4-5], thereby complementing and expanding the range of current genetic control methods (such as sterile insect technique, *Wolbachia*-mediated incompatible insect technique and pathogen interference, and release of insects carrying a dominant (female) lethal gene). However, it will take several years before GD technology is applied to practical management. GDMIs are either in development or have been tested experimentally in laboratories, but none has been assessed in small-scale physically or ecologically confined field trials, or in open release trials.

Concerns

There are concerns that deliberate environmental releases (termed hereafter releases) of gene drive-modified organisms (GDMOs) may eradicate (in contrast to control) the target organism, lead to undesired effects and uncontrolled spread, affect non-target organisms and ecosystems in unanticipated and irreversible ways, or adversely impact biodiversity and health with no current ability for recall [6]. While some adverse effects are similar in many conventional or other genetic control systems, additional concerns are expressed about novel forms of harm. These concerns prompted some scientists, scientific and non-governmental organisations and parliamentarians to call for either a moratorium or strict application of the precautionary principle on GD research. Calls are also made for better understanding of potential ecological and evolutionary impacts of GDMO releases. To address these concerns, recommendations on phased testing, responsible and sustainable deployment, and effective engagement of relevant stakeholders have been developed [5-8]. Moreover, regional approaches facilitating international regulatory oversight and approval have been suggested for governance of GDMOs that may spread across jurisdictional boundaries [9].

Risk analysis

As is the case for any genetically modified organism (GMO), potential releases of GDMOs (i.e. GMOs containing engineered GDs) are subject to risk assessment and regulatory approval in most jurisdictions. In this process, risk assessors provide scientific advice to risk managers on plausible risks that deployment of a GDMO may pose to human and animal health, and the environment. Risks are characterised by testing specific hypotheses on the probability that harm will occur and the severity of harm if it occurs. This process is framed by a problem formulation approach that articulates relevant policy goals, determines criteria for assessing risks and devises tests of risk hypotheses that address those criteria. Decisions on what is an acceptable risk, given potential risk management, and thus if the use of a GDMO should be permitted, is taken by risk managers. This

process would be most efficient where GDMO developers and potential applicants also have a clear understanding of relevant risk concerns and assessment procedures.

Need for new or additional risk assessment guidance?

Risk assessors and managers, along with developers, potential applicants and other stakeholders are currently discussing the need for new or further risk assessment guidance for GDMO releases at many levels. Under the Convention on Biological Diversity (CBD) and Cartagena Protocol on Biosafety (CPB), an *Ad Hoc* Technical Expert Group (ATHEG) on risk assessment recommended development of further guidance on engineered GDs in April 2020 [10]. Among other aspects, AHTEG recognised that existing risk assessment methodology may still be applicable for GDMOs, but specific technical or methodological challenges require further attention. ATHEG's recommendation may ultimately lead to the possible adoption of a decision to develop additional guidance materials for GDMOs at the 10th biannual Conference of the Parties serving as the meeting of Parties to the CPB (COP-MOP10; scheduled second quarter 2021).

To support the European Union in its future work on GDMOs under the CBD/CPB, the European Food Safety Authority (EFSA) assessed whether its previously published guidelines for risk assessment of GMOs are adequate for GDMOs. Focusing on insects (i.e. disease vectors, agricultural pests and invasive species), EFSA concluded in October 2020 that its guidance is an appropriate basis for risk assessment of GDMI releases, but should be more specific to challenges that GDMIs pose [11] (Box 1). For example, guidance on the increasingly important role of modelling in risk assessment for GDMOs [12-13] and post-release monitoring is a particular need, because the temporal and spatial scope of some GDMOs once released precludes testing by observation at such scales.

The World Health Organization is currently updating its 2014 guidance framework for testing of transgenic mosquitoes for use against human disease vectors to incorporate specific considerations for engineered GDs, as some GDs were predicted to broadly impact the multiple phases of testing recommended in this framework [5,7].

Recommendations

Developers and potential applicants need useful and practical guidance to ensure that investment of public and private resources in GD technology is efficiently directed at developing products that can meet acceptable regulatory standards of safety for the environment and health, and receive public acceptance. The development of such guidance is anticipated to entail significant challenges, especially at international level. Due to different, often contrasting, opinions toward GDMOs, and on the adequacy of current risk assessment frameworks for such organisms, defining the scope of guidance, topics to prioritise and procedures to follow for guidance development may be contentious. Moreover, GD technology is evolving very rapidly, and will yield very diverse applications across various organisms. Thus, guidance and derived regulatory requirements can easily be outpaced by scientific advances, especially if requirements are overly prescriptive and aim to encompass all possible GDMO applications. For guidance to be proportionate, useful and practical, it needs to be tailored to the most likely cases moving to practical applications for release (i.e. disease-transmitting mosquitoes whose control is a long-standing public health goal), requiring prioritisation of its scope and regular updates as priority applications change. Guidance should also differentiate between diverse GD systems and refrain from taking single cases to make broader, more general statements that may not be applicable for all GDMOs.

Ideally, guidance should offer an overarching framework that is flexible and outlines general principles and methodology for risk assessment, instead of being prescriptive. Problem formulation, which serves as a starting point for conducting risk assessment, offers such a frame. It involves: (1) identifying protection goals and making them operational in risk assessment; (2) devising plausible pathways to harm that describe how GDMO releases could be harmful; (3) formulating risk hypotheses about likelihood and severity of such events; (4) identifying information needed to test risk hypotheses; and (5) developing plans to acquire new data for hypothesis testing if tests

with existing information are insufficient for decision-making [11, 14-15]. The problem formulation process provides a compelling framework to organise existing knowledge and identify relevant new knowledge on engineered GDs to support case-specific risk assessments and decision-making. Enabling testing of risk hypotheses makes the pathway to harm approach very powerful for risk assessment: harm is defined explicitly, existing information is used effectively, new data are collected with a clear purpose, and risk is characterised against well-defined criteria of hypothesis corroboration or falsification.

Another challenge is the limited direct experience conducting risk assessment of GDMO releases. Nonetheless, principles and methodologies for risk assessment and management, experience from GMO risk assessment, and knowledge from other disease vector and pest control strategies, are relevant to performing GDMO risk assessments. It is important that guidance development involves an iterative process of design, revision and refinement, including review of actual case studies by risk assessment experts and consultation with relevant stakeholders. Once in place, regular review should be continued to establish overall guidance utility and applicability, and assess where any refinements are necessary. This may ensure guidance is realistic and proportionate, and remains consistent with the weight of scientific evidence and familiarity that will be gained with future GDMO releases.

Acknowledgments
We thank Stephanie James for inspiring discussions that helped to develop this publication. We also express our gratitude to the anonymous reviewers for their thoughtful feedback on the manuscript.

Disclaimer statement
The views expressed in this publication are those from the authors and do not necessarily represent the official position of the European Food Safety Authority (EFSA). EFSA assumes no responsibility or liability for any errors or inaccuracies that may appear.

References
1. Alphey, L.S. et al. (2020) Standardizing the definition of gene drive. *Proc. Natl. Acad. Sci. U.S.A.* https://doi.org/10.1073/pnas.2020417117
2. Raban, R.R. et al. (2020) Progress towards engineering gene drives for population control. *J. Exp. Biol.* 223, jeb208181. doi:10.1242/jeb.208181
3. Hay, B.A. et al. (2021) Engineering the composition and fate of wild populations with gene drive. *Annu. Rev. Entomol.* 66, 1. https://doi.org/10.1146/annurev-ento-020117-043154
4. Entomological Society of America (2020) ESA position statement on the importance of continued innovation in gene drive technology. *Ann. Entomol. Soc. Am.*, saaa029. https://doi.org/10.1093/aesa/saaa029
5. World Health Organization (2020) *Position statement: evaluation of genetically modified mosquitoes for the control of vector-borne diseases*, ISBN 978-92-4-001315-5
6. National Academies of Sciences, Engineering, and Medicine (2016) *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*, National Academies Press
7. James, S. et al. (2018) Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: recommendations of a scientific working group. *Am. J. Trop. Med. Hyg.* 98, 1–49. https://doi.org/10.4269/ajtmh.18-0083
8. James, S.L. et al. (2020) Toward the definition of efficacy and safety criteria for advancing gene drive-modified mosquitoes to field testing. *Vector Borne Zoonotic Dis.* 20, 237–251. https://doi.org/10.1089/vbz.2019.2606
9. Kelsey, A et al. (2020) Global governing bodies: a pathway for gene drive governance for vector mosquito control. *Am. J. Trop. Med. Hyg.* 103, 976–985. doi:10.4269/ajtmh.19-0941
10. Ad Hoc Technical Expert Group (2020) *Report of the Ad Hoc Technical Expert Group*, CBD/CP/RA/AHTEG/2020/1/5
11. European Food Safety Authority (2020) Scientific Opinion on the adequacy and sufficiency evaluation of existing EFSA guidelines for the molecular characterisation, environmental risk

assessment and post-market environmental monitoring of genetically modified insects containing engineered gene drives. *EFSA J.* 18, 6297. https://doi.org/10.2903/j.efsa.2020.6297
12. Dhole, S. et al. (2020) Gene drive dynamics in natural populations: the importance of density-dependence, space and sex. *Annu. Rev. Entomol.* 51, 501–531. https://doi.org/10.1146/annurev-ecolsys-031120-101013
13. Golnar, A.J. et al. (2020) Embracing dynamic models for gene drive management. *Trends Biotechnol.* https://doi.org/10.1016/j.tibtech.2020.08.011
14. Roberts, A. et al. (2017) Results from the workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes". *Am. J. Trop. Med. Hyg.* 96, 530–533. doi:10.4269/ajtmh.16-0726
15. Teem, J.L. et al. (2019) Problem formulation for gene drive mosquitoes designed to reduce malaria transmission in Africa: results from four regional consultations 2016–2018. *Malar. J.* 18, 347. https://doi.org/10.1186/s12936-019-2978-5

Additional elements

Table 1. Examples of engineered gene drive approaches in insects, whose design and mode of action are diverse (adapted from [11]; see [1] for definitions)

Intended outcome	Potential for engineered gene drive to spread and persist in target populations[a]			
	Self-limiting (*transient*)		Self-sustaining (*persistent*)[b]	
	High threshold (*localised*)	Low threshold (*non-localised*)[c]	High threshold (*localised*)	Low threshold (*non-localised*)
Population suppression	Split homing-based drives*	Daisy-chain drives*	Underdominance drives [e.g. maternal-effect lethal underdominance*; Medusa*]	Homing-based drives***
	Split rescue drives [e.g. killer and rescue*]		Tethered homing-based drives*	Meiotic interference drives [e.g. X-shredding sex-distorter***]
Population modification	Split homing-based drives [e.g. home and rescue*]	Daisy-chain drives*	Underdominance drives [e.g. reciprocal chromosome translocation***; maternal-effect lethal underdominance***]	Homing-based drives [e.g. home and rescue***]
	Split rescue drives [e.g. killer and rescue***; toxin-antidote recessive embryo*; 2-locus cleave and rescue***]		Tethered homing-based drives*	Engineered Medea and rescue (Medea-like) drives [e.g. cleave and rescue***; toxin-antidote recessive embryo***]

Development status: * Theoretical/conceptual; ** Laboratory proof-of-principle; *** Laboratory proof-of-principle with (some) multigenerational data

[a] Depending on their design and specificities (e.g. split vs. non-split, same locus vs. distant site, DNA target sequence), and fitness costs, some engineered gene drive systems can vary in threshold, and thus fit into different categories

[b] In the absence of mutation or heritable resistance, and assortative mating

[c] Likely hypothetical only because temporal restriction will constrain the engineered gene drive to the vicinity of the release area

Box 1. Risk assessment considerations for gene drive-modified organisms

Non-exhaustive list of considerations for the risk assessment of deliberate environmental releases of gene drive-modified organisms (GDMOs), focusing mostly on insects (i.e. disease vectors, agricultural pests and invasive species), are given below (adapted from [7-8, 10-11]).

- Whether novel aspects of engineered gene drives (GDs) as compared with naturally occurring GDs and genetic and classical biological control methods present new hazards, and may introduce additional factors into risk assessment should be assessed on a case-by-case basis;
- Experience from current insect disease vector/pest control strategies can inform GDMI risk assessments, though caution is required as the specific control systems compared differ in various aspects;
- Understanding how engineered GDs may spread and persist in target populations in the field is crucial for risk assessment;
- Resurgence of an intrinsically harmful target organism due to failure of an engineered GD or resistance to either the GD or its cargo/payload genes could cause harm as is the case for any other disease vector/pest control strategy;
- Intended and unintended "on-target" and unintended "off-target" sequence modifications caused by site-directed nucleases can affect fitness. Such effects may be more important for population modification where the genetic modification must remain present and active at high frequency in target populations over long periods of time, whereas in suppression systems the frequency would decline over time as the population falls;
- *In silico* analyses help to identify potential "off-target" effects in target populations, but caution is required when interpreting such data, as they are subject to limitations (i.e. natural population heterogeneity). "Off-target" activity can also be addressed through the identification and monitoring of fitness and other phenotypic changes;
- Continued assessment and monitoring of genetic and phenotypic stability may be needed over multiple generations under confined conditions prior to deliberate environmental release, and in the field after release as part of post-release monitoring;
- Greater use of mathematical models is anticipated to address the long temporal scale and wide spatial scale of specific GDMO applications, and extrapolate data gathered from confined experimental systems to field conditions;
- The comparative risk assessment paradigm for living modified organisms, which uses the case-by-case principle and an iterative, stepwise/staged/tiered testing approach, and considers different lines of evidence, including mathematical modelling, in a weight of evidence approach, may still leave some uncertainty before open field testing or field implementation of some GDMIs. It is therefore important that post-release monitoring is scientifically designed and implemented;
- Gathering relevant data for self-sustaining and low threshold GDs in open field trials may be challenging due to their spatially and temporally unrestricted nature and current inability for recall. Besides gathering data under confined conditions, potential applicants may wish to consider the utility of prior field testing of a related self-limiting strain as an intermediate step to reduce uncertainties in risk assessment;
- There will often not be a single comparator (i.e. non-genetically modified insect with a genetic background as close as possible and relevant to that of the genetically modified insect), but a range of relevant comparators to inform risk assessment and contextualise risks;
- Mitigation and management plans to ensure that risks are at acceptable levels should undergo systematic evaluation in a manner similar to risk assessments.