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Risk assessment challenges of synthetic gene drive organisms

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Introduction

The development of gene drive organisms (GDOs) is highly controversial, as illustrated by the intense academic, political and societal debates over their potential deployment for a variety of applications from public health to conservation, agriculture and dual-use technologies.

The controversy stems from the biological and conceptual novelties of GDOs (Simon et al., 2018). Unlike with current genetically modified organisms (GMOs) released into the environment for commercial or medical use, gene drives are designed to purposely spread genetic modifications through entire populations. The capacity for spread and persistence makes them attractive to developers, but distinct from GMOs released to date. Even for GMOs with which there is some experience, gene flow or contamination has mostly been considered a risk to be avoided. GDOs make such risks certainties.

As currently envisioned, the "driving" of a genetic trait through a population is achieved by inserting transgenes into an organism that encode for the genetic engineering (GE) machinery, another key distinguishing feature of most gene drives. Essentially, "the laboratory moves into the environment" (Simon et al., 2018). Whereas before, GMOs were genetically engineered in the laboratory and then released into the environment, GDOs are engineered in the laboratory to carry and spread the genetic engineering machinery (e.g., CRISPR/Cas9 genome editing machinery) to other organisms or to future generations, carrying out genetic engineering in each generation for perpetuity. As further described by Simon et al. (2018), "gene drives imply a shift from the release of a finished and tested product to the release of an adjustable tool for genetic modification that is released into ecosystems". Consistently, gene drives will likely require dozens of generations to establish the desired effect in the target populations (Oye et al., 2014), requiring repeated genetic interventions including DNA cutting and insertion of genetic sequences, with a broad range of specific and unintended next-generation effects.

Intended applications to modify wild, self-propagating populations, as opposed to cultivated, annual genetically modified (GM) agricultural crops, also introduce increased complexity and unpredictability to any risks of GDO releases into the environment. GDOs thus represent an example of an emerging trend to modify populations in

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the wild, facilitated by advances in GE techniques (Heinemann and Walker, 2019; Heinemann, 2019; Sirinathsinghji, 2019). Unintended effects thus cannot be fully assessed prior to release, as any deployment, even as part of a field trial, is effectively an open release that is persistent and irreversible by design, with the capacity to spread beyond the initial area. Modifying disease vectors that transmit diseases with complex epidemiologies influenced by ecological factors, adds yet another layer of complexity, with potential implications for human health.

Such novel features raise urgent and critical questions for risk assessment and risk management. As expanded below, the nature of GDOs creates novel risks, while our understanding of any potential adverse impacts on the environment or human health is limited by critical knowledge gaps and uncertainties with regard to both intended (e.g., population eradication) and unintended (e.g., spread to non-target species, knock-on effects on disease epidemiologies or ecosystems) effects. Current risk assessment and risk management protocols are understandably inadequate to address the evolving nature of GE technologies, in particular gene drives, and cannot simply be accommodated or extrapolated from their current focus on existing GMOs (see Then, 2020). Indeed, there are concerns even with current procedures of risk assessment of existing GMOs (Mueller, 2019; Heinemann et al., 2013; Bauer-Panskus et al., 2020). It is thus imperative that adequate governance and regulation for GDOs are put in place well before any potential environmental release takes place, and serious assessment of whether release should even occur is needed.

Recognising the sobering potential for adverse effects, a decision by the 2018 Conference of the Parties to the Convention on Biological Diversity (CBD) placed strict conditions for the consideration of any environmental release of GDOs, including for experimental releases and research and development purposes. These conditions included the need for risk assessment and the application of the precautionary approach, along with the requirement for full, prior and informed consent from potentially affected indigenous peoples and local communities. The precautionary approach, as referenced in the decision, includes the right to take precautionary measures where there is a lack of scientific certainty of safety. As outlined below, it can be argued that the current state of knowledge is not adequate to resolve the significant risks and uncertainties that gene drives present.

Current state of gene drive organism development

Gene drives can be genetically engineered into any kind of organism or infectious agent. The assessment of the risks of gene drives requires competence in the biology of the organism(s) targeted and the drive agent itself. To date, the focus of the biosafety community has been on the use of drives in sexually reproducing organisms and that is the focus of this briefing. However, in the future other kinds of drives may become important. Furthermore, many organisms that reproduce sexually may also reproduce asexually at times. This ability to swap reproductive processes has implications for potential risk mitigation strategies.

The first genetically engineered gene drive in a multicellular organism was demonstrated in 2011 in *Anopheles gambiae* mosquitoes (Windbichler et al., 2011). Since 2014 and the introduction of CRISPR-based gene drive techniques to organisms other than bacteria, developments have accelerated, with demonstrations in yeast (e.g., DiCarlo et al., 2015; Shapiro et al., 2018; Basgall et al., 2018), flies (e.g., Champer et al., 2017; Champer et al., 2019a), mice (to partial effect) (Grunwald et al., 2019), *Anopheles gambiae* again (Hammond et al., 2017; Kyrou et al., 2018; Simoni et al., 2020), *Anopheles stephensi* (Gantz et al., 2015), and also *Aedes aegypti* mosquitoes (Li et al., 2020). CRISPR-Cas genome editing techniques have become the genetic "drive" tool of choice due to their technical ease and flexibility, but other systems such as using selfish genetic elements, meiotic drivers and underdominance are also being developed. Improvements in preventing resistance development against GDOs are a major aspect of research and development. A recent publication showed a lack of resistance development in mosquitoes and an efficacious crash of laboratory populations by targeting conserved sequences that the target insects could not evolve alternatives to at the size of the experimental population (Kyrou et al., 2018).

The vast majority of GDOs are designed to cause unlimited spread of genetic modifications, termed "global" or "self-sustaining" gene drives. Global gene drives have been suggested for applications including disease vector eradication projects such as the Target Malaria project. A limited number of gene drives are being designed to be "local" or "self-limiting", with limited spatio-temporal spread. Such in-built limiting designs are largely lacking in empirical demonstration, and research and development is lagging behind that on global drives, though a few early examples have been

published (e.g., Li et al., 2020 in *Aedes* mosquitoes; Noble et al., 2019). Claims of controllability and reversibility of gene drives are thus speculative at this stage. Meanwhile, whereas self-limiting designs are being developed, global gene drives are being actively promoted as the first potential application – for tackling vector-borne disease. Indeed, in many cases a gene drive would need to be global to even be envisaged as an effective tool to tackle diseases such as malaria.

Gene drives are being further divided by application type, including "population suppression" (intended to eradicate populations) and "population modification" (intended to spread a desired trait in a population, such as resistance to pathogens) strategies. However, as recently demonstrated, such distinctions may indeed be more theoretical than practical, with unanticipated outcomes. So-called population modification GDO mosquitoes were recently demonstrated to cause extinction of a laboratory population within three generations due to knowledge gaps with regard to the target gene (Pham et al., 2019). At the same time, "global" gene drive and wild-type populations have been shown to stably co-exist (Champer et al., 2019b; Price et al., 2019). Such theoretical distinctions are not currently sufficient to suggest that some forms of gene drives are "safer" for release than others or could act as a form of risk management strategy. Any narrowing of risk assessments based on technical characteristics of different gene drive mechanisms may thus be inadequate in detecting potential unintended outcomes.

While a wide range of proposals for GDOs have been suggested and these GDOs are at various stages of development, insects are increasingly becoming the main target of gene drive applications, including wasps, fruit flies, kissing bugs, red flour weevils and the Argentine stem weevil. However, applications are in development or suggested for various other species including plants (amaranth), mammals (mice, rats, stoats, possums, cats), fish (lionfish), birds (starling), nematodes and snails (CSS, ENSSER and VDW, 2019).

Considerations regarding molecular characterisation

Unintended effects of genome editing systems are now well established and include both unintended on- and off-target effects such as mutations (e.g., deletions and complex rearrangements (Kosicki et al., 2018)), chromosomal recombination events (e.g., Brunner et al., 2019, documented in insects), high-frequency production of aberrant protein products (e.g., Tuladhar et al., 2019), even for gene knock-out targets (Smits et al., 2019), multiple integration events (Skryabin et al., 2020), and unintentional incorporation of foreign genetic material, creating unintended transgenic organisms (e.g., Ono et al., 2019). These unintended effects were observed in the laboratory when generating genome edited organisms, but might remain undetected if the laboratory is taken into the wild.

Such unintended genetic and proteomic changes could alter the biochemistry within the target organism, with unknown implications. For example, increased levels of toxins or allergens, or novel toxins or allergens could be produced that may affect predator species or prey (e.g., increased levels of immunologically active compounds in the saliva of a GDO mosquito) and its capacity to transmit disease, or alter the behaviour of an organism. Such unintended effects are not restricted to CRISPR/Cas systems, but to all techniques that induce double-stranded breaks.

Unintended molecular effects can vary with differing genetic backgrounds (Canver et al., 2018), presenting a particular challenge for GDO risk assessment. This is distinct from current commercial GM plants where they are introduced into genetically uniform varieties that are harvested annually. While the information remains incomplete, the genetic diversity of suggested target species such as *Anopheles gambiae* and *coluzzii* mosquitoes is thought to be high (Anopheles gambiae 1000 Genomes Consortium, 2017). Increased vigour in GDO populations may also occur, as previously noted with GM mosquito releases (Evans et al., 2019).

Unintended effects may also lead to the development of gene drive resistance. While resistance may render the gene drive inactive and thus no longer able to spread at intended rates, the genetic modification will still be present and could indeed get fixed in the population. As such, resistance cannot be considered a form of confinement or mechanism to limit spread. While it is considered that mutations often result in fitness costs, it is also conceivable that any mutations resulting from active or even indeed inactive gene drives that have developed resistance, could also increase fitness. As experienced with first-generation GMOs, unexpected effects on fitness have already been documented when crossed onto other genetic backgrounds, e.g., rice to weedy relatives or with oilseed rape outcrossing to conventional varieties (reviewed by Bauer-Panskus et al., 2020).

Such complexities render current risk assessment protocols inadequate for assessing the molecular effects of drives released from one organism into wild populations. In addition, after release, the gene drive may change, either because it mutates or because of changes in the genetic composition of recipients or changes in environmental conditions. Any risk assessment of these effects is thus determined by the spatio-temporal dimensions of gene drive persistence and number of crosses with wild populations.

Spread and persistence

A prerequisite for global gene drives is their ability to spread and persist, raising numerous implications for any risk assessment and risk management process. Even imperfect gene drives have been predicted to be "highly invasive", potentially spreading from the release of just two or more individuals (Noble et al., 2018). Highlighting the significant implications of gene drive technology, leading gene drive mosquito developers described in their initial publication that the technology "could be used to take the step from the genetic engineering of individuals to the genetic engineering of populations" (Windbichler et al., 2011).

The ability to spread without further human intervention is the design intention, but that ability raises biosafety concerns at the ecological level. The less control there is on spread, the greater the potential to unintentionally eradicate or alter target and non-target populations. Gene drives may spread without contributing to fitness, a phenomenon with which biologists have relatively little experience and therefore are limited in their power to predict outcomes.

Several factors may affect the ability or rate of gene drive spread within its target species – as well as potential outcrossing or spread to non-target species – such as gene flow, population structures, resistance, fitness, dispersal patterns, species barriers, ecological interactions and mating behaviour. Further, with gene drive designs focusing on preventing resistance development by, for example, targeting conserved sequences that are also present in non-target organisms, the likelihood of establishment is further increased.

As recognised in a report from the US National Academies of Sciences, Engineering, and Medicine (NASEM, 2016), modelling the spread of GDOs is a prerequisite for any GDO release. However, currently such approaches are unfeasible due to knowledge gaps on the above parameters. Beyond that, there is the potential for unexpected next-generation effects. Unintended effects at the molecular level can affect such parameters which may occur generations after assessment and release, making it extremely difficult, if not impossible, for modelling to comprehensively predict risk.

Eradicating/altering entire populations

In order to assess the safety of using gene drives to eradicate or alter populations, knowledge on a species' role in ecosystems, its place in the food web, mating behaviour, feeding behaviour and its genetic diversity, is required.

Ecosystem or food web effects are difficult to study, interpret and understand due to the high level of complexity (Bøhn, 2007). As experienced with invasive species, altering one level of the food web necessarily impacts on other levels, resulting in both direct and indirect effects that tend to have a lag phase and thus may take decades to become visible. For example, with the invasive vendace fish experience in Norway, there was a time delay of 35-40 years from the first introduction to the observation of the first ecosystem effects. In Australia, it was only after 20-30 years that the adverse ecological effects of rabbit introductions became visible (Bøhn, 2007). While invasive species have to adapt to new regions or ecosystems however, GDOs would instead be spreading within populations already adapted, so some differences in ecosystem dynamics would be expected.

Potential target species may play numerous ecological roles such as providing a food source, but critical knowledge gaps remain regarding the extent of a species' role/s and interactions. A case study on the GM olive fruit fly highlights such complexities that would need to be considered for any gene drive application, with numerous parasitic, symbiotic, predation and prey interactions that are distinct to each life-stage (Preu et al., 2020). Mosquito disease vector applications serve as another example of knowledge gaps and uncertainties regarding ecological roles, but experts warn of unintended effects on target and non-target species, with potentially serious ecological impacts (Hochkirch et al., 2018). Mosquitoes in general have been suggested to be particularly sensitive to unforeseen effects due to numerous unknowns such as how vectors and their pathogens would react to the modified traits, and the ecological uncertainties of disease epidemiologies (Beisel and Boëte, 2013).

Considerations for public health interventions

In the case of disease vector GDO applications, potential unintended outcomes such as disease-rebound effects following a temporary suppression/replacement are a serious concern. Such a scenario may arise from unintended molecular effects such as resistance development to the spread of the gene drive construct, or other mechanisms such as disease resistance to a gene drive modification strategy, recovery of populations following local/regional suppression, or through niche-replacement with alternate disease vectors.

There are about 450 species of *Anopheles* mosquitoes, 70 of which transmit malaria. Similarly, there are approximately 80 species of *Aedes* mosquitoes that transmit disease. The effects of targeting a limited number of vectors on overall disease transmission thus remain a complex question. Rebound effects have occurred following cessation of pesticide programmes, with devastating effects resulting in huge losses of life (Packard, 2007; Romi et al., 2002). Other unpredictable effects have already been experienced or flagged with current mosquito control methods such as sterile insect techniques, where there are indications of an increase in neighbouring populations outside release sites, or indeed within release sites, due to reduced larval density (Yakob et al., 2008).

Further, as Beisel and Boëte (2013) warn, the eradication of populations by GM (including GDO) mosquitoes requires the coexistence of people and mosquitoes to enable the spread of the modified trait. They highlight that this logic is "in direct opposition to current means of vector control which focus on the avoidance of bites, repellence and reduction of the mosquito population". The reliance on people's willingness to get bitten for GDO establishment and spread means that GDO mosquitoes and other current public health interventions may not be able to coexist. How such disease parameters and existing public health interventions are incorporated into any risk assessment and risk management process is a critical challenge for protecting human health.

The evolution of resistance of pathogens to gene drive traits has also been suggested to be a likely consequence of gene replacement strategies where the gene drive trait is designed to block disease transmission (Bull et al., 2019). Low expression of the gene drive constructs may also aggravate it, a factor that may be influenced by changing genetic backgrounds interacting with changing environmental conditions.

With suggestions that simultaneous or sequential release of multiple GDOs may be needed to delay resistance and maintain efficacy (e.g., North et al., 2019), as well as multiple target species existing for any particular application, risk assessment has to then also consider potential interactions of multiple GDOs and any potential combinatorial effects.

Considerations for eradicating invasive species

Gene drives have been proposed to eradicate invasive species. However, even for invasive species, experts in the field warn of potential detrimental ecosystem effects (Kopf et al., 2017). Invasive species can have unexpected functional roles in food webs, habitat provision or ecological support functions, as well as in biophysical changes such as loss or alteration of habitat, or modification of an ecological process (e.g., nutrient transfer, sediment stability, seed dispersal or pollination (Schlaepfer et al., 2011; Zavaleta et al., 2001)). An extreme example is the eradication of cats from the "World Heritage Island" Macquarie Island in the sub-Antarctic, which resulted in serious ecosystem degradation as a result of the consequent increase in rabbits despite control measures in place (Bergstrom et al., 2009). It is thus pertinent to note that risk assessments limited to so-called keystone or valued species may not encompass potential effects on biodiversity as a whole. Further, any suggestion that an environmental risk assessment for targeting invasive species would be unnecessary (e.g., EFSA, 2019), or would require less stringency, fails to take into consideration such uncertainties and unpredictability.

A more obvious concern of removing an invasive species from its non-native environment, e.g., rodents from New Zealand, is the potential escape and spread of that organism to native habitats. In addition to ecological concerns, such a scenario raises political questions regarding who would be responsible for conducting a risk assessment and any subsequent authorisation, and who would be responsible for any liability and redress in case of adverse effects if a gene drive target organism spreads to its native habitat.

Methodological problems with gene drive risk assessment and risk management

The novelties of gene drives raise numerous meth-

odological problems for risk assessment (Then, 2020). As Then (2020) highlights, just as experience gained with conventional breeding cannot be extrapolated to existing GMOs, experience gained with such GMOs cannot also be directly extrapolated to assessing GDOs. Current risk assessment is already widely criticised for its limited assessment of potential risks of existing GM crops and animals (Bauer-Panskus et al., 2020).

Indeed, to date, focus on assessing molecular concerns is arguably of limited value when next-generation effects are taken into consideration. Genetic stability or fitness, for example, can only be calculated in regard to the strains used in the laboratory and under defined conditions. However, it hardly can be calculated prior to release, as this is dependent on environmental and future changes, with potentially evolutionary consequences. Modelling can only take into consideration known interactions and characteristics and is thus insufficient to predict all potential outcomes.

Altering or eradicating populations also raises fundamental issues with regard to assessing effects on potential receiving environments, given that GDOs are designed for wild populations. Receiving environments will not be static over time and space. Therefore, it is also not possible to fully simulate the receiving environment in laboratory tests or via modelling, considering the complexities of ecological effects that may result from eradicating or replacing entire populations. For the first time in a GMO risk assessment context, it will also require assessing effects of removing a population or even a species, instead of just the effects of introducing a novel GMO.

The current comparative approach in risk assessment that compares the transgenic versus conventional non-transgenic counterparts for both intended and unintended effects, is another clear challenge. Assessing effects on wild populations where GDOs are introducing changes to population dynamics over time by altering inheritance patterns makes the utility of comparison to wild relatives limited. As highlighted by Then (2020), the current European Food Safety Authority (EFSA) guidelines (2013) and European Union (EU) regulations, even with existing animal GMOs, acknowledge increased uncertainties, with variations occurring over time, long-term effects due to spatio-temporal complexities, and other ecological factors such as the inability to simulate a receiving environment in the laboratory. Nonetheless, GDO proponents have leant on the argument that GDOs could be compared to other techniques; for example, with specific applications such as mosquitoes, comparisons are being drawn to *Wolbachia* bacterial infections or sterile insect techniques. However, *Wolbachia,* whilst able to spread through populations, does not involve generations of crossing of genomes and GE processes that intervene at the genetic level required for gene drive establishment (Oye et al., 2014), making such comparisons of limited relevance.

With regard to the phased-testing approach used in risk assessment, field trials of GDOs are not possible due to the inability to recall the organisms once released. While island locations have been suggested as potential trial site locations (e.g., Lukindu et al., 2018), they are insufficient as a containment measure, as recently acknowledged by the CBD's Ad Hoc Technical Expert Group on Synthetic Biology (2017): "Islands are not ecologically fully contained environments and should not be regarded as fulfilling the conditions in the definition of contained use as per Article 3 of the Cartagena Protocol unless it is so demonstrated." It has likewise stated that "the step of release into the environment might be irreversible".

Even research at the laboratory stage requires further oversight. Currently, there are no specific international rules on contained use of GDOs. Strict conditions are warranted, considering their potential to spread and persist from the release or escape of a few organisms (Noble et al., 2018). Multiple strategies need to be implemented as it is possible that "any single confinement strategy could fail" (Akbari et al., 2015), including molecular, ecological, reproductive or physical measures. Current contained-use measures, as applied to pathogens, may include some that are not relevant for GDOs, and others that may not provide adequately for the suite of controls necessary to contain them, but may serve as an initial guide to developing contained-use standards (Lim and Lim, 2019). Indeed, GDOs exhibit some of the same traits of pathogens that justify stringent measures, including spread, persistence, irreversibility and, with population suppression methods, lethal traits. This means that there is a need to adapt the details accordingly, along with an additional focus on potential environmental hazards due to potential species and ecosystem effects (Simon et al., 2018).

The inability to recall GDOs also calls into question the rationale behind monitoring and detection of organisms following release. Currently, remediation strategies remain theoretical. Rescue drives still suffer many technical and biosafety chal-

lenges, cannot revert populations to wild type, as a drive will take several generations to establish itself (Frieß et al., 2019). Suggested mitigation strategies for mosquito applications also include large-scale pesticide spraying (James et al., 2018). However, the rationale for GDOs is to address the limitations of current interventions that have failed to eradicate target species to date, such as pesticide use on mosquitoes. Further, with GDOs being purported to deal with rising pesticide resistance, such a strategy will fail in the event of introgression into pesticide-resistant populations. In the event of monitoring and detecting GDOs in the environment, detection at the genetic level of both inactive and active constructs would require detailed sequencing protocols respectively. Ecological effects would need to be monitored for both intended and unintended effects. Uncertainties surrounding outcrossing to non-target organisms raise further challenges. Fundamentally, where there is a likely chance that GDOs cannot be retrieved, the only way to ensure a release is safe, is to *not release* it in the first place (Then, 2020).

Crucially, uncertainties with regard to next-generation effects, and genetic by environmental interactions in self-propagating wild populations raise "known unknowns" and uncertainties. Then (2020) suggests a "cut-off criterion" analogous to that used for EU chemical substance regulation (EU Regulation 1907/2006), and regulations (EU Regulation 1107/2009) on persistent organic pollutants including pesticides, where fate and behaviour in the environment is taken into consideration alongside toxicity. As stated by Then (2020), "the level of uncertainties might increase to an extent that the delicate balance between knowledge and non-knowledge is distorted allowing tipping points to be reached in risk assessment, if inherent non-knowledge increases to an extent that robust risk assessment is disabled."

As such, in order to operationalise the precautionary approach, there is a need to incorporate mechanisms such as cut-off criteria, based on spatio-temporal controllability, into GDO risk assessment. This means when such cut-off criteria are met, no releases should occur, providing a means to efficiently facilitate decision-making by sufficiently integrating uncertainty and knowledge gaps into the process. Then (2020) recommends a number of parameters that can be incorporated into the criteria including (1) the biology of the target organism, (2) its known interaction with the environment, and (3) the biological characteristics of the GDO.

Conclusions

The current state of knowledge of gene drives exposes fundamental uncertainties and scientific knowledge gaps that prevent conduct of robust and reliable risk assessments. As such, it is vital that new approaches are developed that can sufficiently incorporate such uncertainties and the fundamental state of "non-knowledge" over potential implications of GDO releases. Approaches such as cut-off criteria based on spatio-temporal controllability provide a mechanism by which risk assessments can adequately consider and operationalise the precautionary principle at an early stage in the assessment process. However, decision-making on gene drives must also be widened to incorporate the breadth of factors that may be affected by GDO releases, with implications going beyond potential direct effects on biodiversity and human health.

As widely highlighted, the novel characteristics of spread, and the depth of intervention that gene drives permit, along with the suggested applications into serious issues such as public health necessitate a debate that goes beyond merely risk assessment. Of crucial importance is that mechanisms are in place for full public participation in the decision-making process, that potentially affected people have control over the decision-making process and their full, prior and informed consent is operationalised. The rights of affected communities to make such decisions must be respected (Meghani, 2019).

Such broader processes need to consider issues of cost-benefit analyses and alternative solutions, particularly considering how gene drive projects may restrict or hamper development of sovereign solutions to issues such as disease control. As highlighted by independent scientific organisations (CSS, ENSSER and VDW, 2019), gene drive projects have thus far been subject to science-based hype and have the potential to divert resources away from other, less risky approaches. Further, it is vital that discussions move away from solely paving the way for gene drive approvals, and that "genuine empowerment of all affected parties in the interests of making better choices must not be conducted on the premise that the technology will be accepted. Choices of alternative pathways of development for the future must be available" (CSS, ENSSER and VDW, 2019).

Indeed, as recalled in *Late lessons from early warnings: the precautionary principle 1896-2000* (Harremoës et al., 2001), there is no evidence that the

application of the precautionary principle restricts innovation; indeed, it can instead foster innovation in other, less risky directions. It is thus imperative that the precautionary principle is applied to gene drive technologies in order to safeguard biodiversity and human health and provide genuine opportunity to find the most appropriate and effective solutions for the issues gene drives purport to address.

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