mathematical ecology

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Ecological Risks of Gene Drive Technologies

Overview

- Gene drive technologies offer enormous promise to improve human, animal and environmental health, yet also entail potential ecological risks.
- There is a vast array of potential uses of gene drives, over a huge range of species, environments and goals (Figure 1).
- Current regulation of genetically modified organisms provides useful precedents, but has some gaps in relation to gene drives.
- We recommend a coordinated international effort to produce overarching principles for environmental risk assessments, supported by more detailed guidance for different applications.
- At national level, a campaign of education to raise awareness among researchers and biosafety/bioethics committees should be accompanied by a database of decisions on contained use, to share good practice and promote consistency.

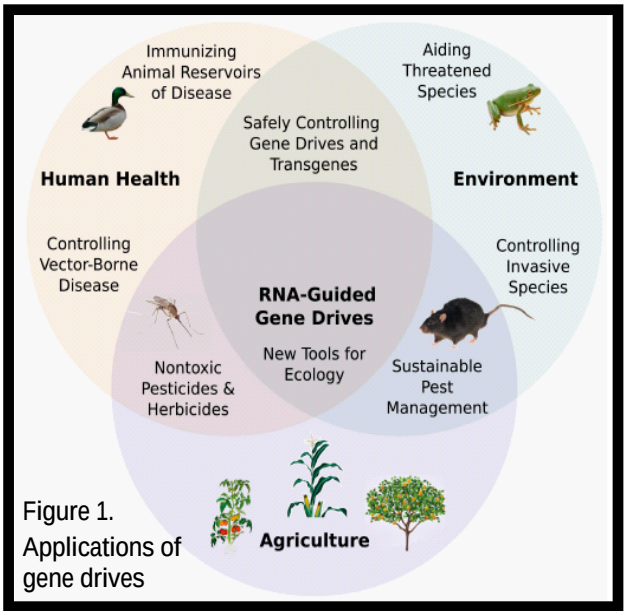

Figure 1. Applications of gene drives

Esvelt et al (2014) eLife 3:e03401 [1]; Figure 7 reproduced under Creative Commons licence, http://dx.doi.org/10.7554/eLife.03401.014

Background and introduction

In sexually reproducing organisms, most genes have a 50:50 chance of being inherited by each of the organism's progeny. This is known as Mendelian inheritance, after Gregor Mendel, whose 19th Century experiments demonstrated the laws of heredity. Gene drives are genetic mechanisms by which a gene (or genetic element, a portion of DNA) is inherited by more than half the progeny; this biased transmission, even if it is harmful to the organism, can allow the gene to spread or 'drive' itself through a population, and gives rise to the name 'selfish genetic element'[2]. Gene drive mechanisms of diverse kinds exist in nature [2,3]. Molecular and synthetic biology are now sufficiently advanced that engineered gene drives can be created, with increasing ease and scope[4].

Broadly, synthetic gene drives can be partitioned into two categories according to the nature of the trait that they drive into a target population: 'suppression drives' reduce the population of the target species (for example by damaging a gene with a function essential to survival or reproduction); 'modification drives' (or 'replacement drives') introduce a desirable trait (such as reduced capability to transmit a pathogen, or lower resistance to existing control measures) that is intended to persist in the population. Potential applications span human and animal health, agriculture and conservation (Figure 1), and include[1,5]:

- eliminating populations of arthropods, e.g. mosquitoes, that transmit (or 'vector') human, animal or plant disease;
- rendering vector populations unable, or less able, to transmit a pathogen (this approach is harder to achieve than suppressing the population, but the vector population would remain in its ecological community);
- protecting threatened species by eliminating or modifying populations of disease vectors, or by modifying the threatened population to resist harm from natural enemies;
- protecting human health by rendering animal reservoir populations immune to a pathogen;
- controlling damaging invasive species;
- enhancing sustainability of pesticides and herbicides by driving susceptible genes through populations that have become resistant;
- engineering pests or weeds to be vulnerable to a harmless molecule that can then be used as a non-toxic pesticide or herbicide.

Issues arising from new technology

In 2016, the National Academies' report *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*[3] remarked on the breath-taking pace of change in gene drive research and asserted a need for ecological risk assessment in the authorization of gene drives for environmental release, given their ability to persist in the receiving environment. Although there is a growing body of research on the molecular biology of gene drive systems, research addressing the ecology of gene drives lags behind and there are considerable knowledge gaps. Research on population dynamics, evolutionary processes and ecosystem dynamics will be essential. The report identified knowledge gaps around how a gene drive's effectiveness is affected by factors such as:

- fitness costs - reduced ability of the organism to reproduce or of its offspring to survive to reproductive maturity;
- dispersal, and gene flow within and between populations – through interbreeding and mating with immigrants;
- mating behaviours (e.g. variation in courtship activities);
- population sub-structures (e.g. mix of ages, or sexes, or spatial structure); and
- generation times.

The report concluded that, under the current regulatory framework in the USA,the environmental assessment and the environmental impact statements required by the National Environmental Protection Act, are incapable of properly characterizing, and capturing, the risks of gene-drive modified organisms. It made recommendations for development and application of appropriate 'ecological risk assessment tools'.

In this policy note we focus on ecological considerations. An effective ecological risk assessment should identify 'hazards' and accurately predict the 'risks' of harmful effects arising from those (Box 1).

Gene drives and gene editing

Current applied research adapts natural gene drive systems found in microbes. With one exception (*Wolbachia*, see below), the focus is on mechanisms based on endonucleases, i.e. gene products that cut a particular sequence of DNA.

Homing endonuclease genes, or HEGs, generally confer no advantage on the host organism, but spread themselves through populations, by

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Box 1. Risk assessment

'Hazards' are substances or activities with potential to cause adverse effects (however likely or unlikely) to living organisms or environments[6].

Risk assessment entails[6]:

- hazard identification – environmental release of a gene drive;
- hazard characterisation (identifying potential harms that might occur) – e.g. creating new or more vigorous pests and pathogens; exacerbating the effects of existing pests through hybridization with related transgenic organisms; harm to non-target species, such as soil organisms, insects, birds, and other animals; disruption of biotic communities, including agro-ecosystems; irreparable loss or changes in species diversity or genetic diversity within species[3,7];
- assessment of exposure (of the population, species, habitat or ecosystem to the hazard) – dispersal, gene flow and ecological interactions;
- risk characterisation - probability of a harmful effect occurring given the nature of the hazard and the extent to which people, animals, plants and/or the environment are exposed[6]. In one common formulation *risk* = *hazard* x *exposure*.

cutting DNA and using a cellular repair mechanism to copy themselves into the 'empty' chromosome in individuals that carry the HEG on only one chromosome of a pair (Box 2). Female mosquitoes transmit disease when feeding on blood (male mosquitoes do not bite). Researchers have inserted HEGs in *Anopheles gambiae* mosquitoes, the major vector of human malaria, to reduce their capacity to transmit disease[8,9]. These HEGs were adapted from nature and required years of work to create a synthetic version that targets a desired DNA sequence and is positioned appropriately in the genome[9]. One HEG cuts DNA during sperm formation, so sperm that make daughters have their DNA 'shredded' by multiple cuts, while sperm that produce sons are unaffected; in experiments this resulted in 90% of progeny being male, rather than the Mendelian 50%. Another approach places a HEG within a gene that is essential for female fertility, so that successful homing – copying the HEG into the normal version of the gene on the other chromosome – knocks out that essential gene. Both these strategies result in fewer female mosquitoes to lay eggs, bite people and transmit disease.

CRISPR (Clustered regularly-interspaced short palindromic repeats) are segments of DNA from bacterial immune systems. An accompanying protein, such as Cas9 (CRISPR-associated protein 9), acts as molecular scissors that cut DNA at a target sequence specified by a small guide molecule (RNA). This system can be designed to target almost any specified sequence of DNA in a genome, to insert, edit or disrupt genes (Box 2), and researchers have done so in a range of organisms from yeast to monkeys, and in human cells[4]. The ease with which CRISPR systems can edit genomes has led to calls to use such methods responsibly, especially as researchers might create gene drives inadvertently[10,11].

Wolbachia are maternally-inherited parasitic bacteria that spread selfishly through insect populations by favouring offspring of *Wolbachia*-infected females. Transferring *Wolbachia* to mosquitoes reduces their ability to transmit some human pathogens[13]; this is being tested as a gene drive to spread that transmission-reducing trait through wild populations[14].

Gene drive should not be confused with genetic modification (artificially altering genetic material); they overlap but are not the same. Genetically modified (GM) plants or insects currently in the field have no gene drive, the transgenes are inherited at Mendelian rates; conversely, *Wolbachia* gene drive is not GM. Regulations for GM crops are well established.

Population-suppressing GM insects are in trials for mosquitoes[15-18] and for agricultural pests[19-21], and regulations are evolving. In the USA, requirements depend on the species and trial site, and include environmental assessments by the Department of Agriculture (for agricultural pests)[22] or the Food and Drug Administration (for mosquitoes)[23]. The *Wolbachia* gene drive, lacking any genetic modification of the release organism, requires no compulsory risk assessment prior to release in many jurisdictions; voluntary assessments of risk have been published[24].

While GMO regulations provide some precedents, new or distinct features of gene drives pose additional challenges. The CRISPR-based molecular technology is very easy and quick to use, and has a vast range of potential targets (at all scales - DNA sequences, species, and objectives). Current GM insects contain self-limiting constructs, which because of their disadvantages to the insects quickly disappear from the population unless large-scale releases are sustained. In contrast, a self-sustaining gene drive might spread through an entire population, and possibly beyond, potentially from a single small release or escape. Current regulations were designed for managed populations (agricultural settings for engineered plants or modified insect pests), but gene drive applications are typically designed for wild populations, which may change matters to be considered and their priority or weighting.

Contained use – escape risk

The primary ecological concern is spread of a gene drive through one or more populations, possibly an entire species, following inadvertent release. Modified individuals could escape from a laboratory, or from a 'contained' trial. This includes invasion into other lab stocks, which might not be managed as securely as the gene drive strains, as well as into wild populations.

The consequences of escape could include:

- individual-level phenotypic (novel trait) effects;
- population/species-level effects;
- wider ecological consequences;
- effects on public confidence in science, genetic technology and its regulation.

Biosafety / bioethics committees need to be aware of the potential risks relating to gene drives. The CRISPR molecular technology makes it too easy to create a gene drive unintentionally, and these pose the highest risk of escape into the environment because researchers will be unaware of the need for containment. Steps should be taken to encourage researchers to submit all proposed CRISPR-based genetic experiments to the committee, and further detailed consideration given where the molecular design is such that a gene drive mechanism might be created.

With such measures in place, current regulations and guidance for contained laboratory use of GM organisms are reasonably appropriate for gene drive systems. The main focus for improvement should be on:

- Education about gene drives, to raise awareness among scientists;
- Guidance for biosafety committees on risk assessments;
- Making biosecurity decisions on gene drive research openly available, to promote consistency and facilitate calibration against other studies and between committees.

Box 2. Harnessing DNA repair

All organisms have mechanisms to repair or destroy faulty molecules. When DNA is damaged by a break or cut, the cell's machinery has broadly two methods to repair it.

One is to re-join the two broken ends, known as non-homologous end-joining (NHEJ). NHEJ is error-prone and often imperfect, e.g. omitting DNA bases or joining two ends from different breaks, and can result in disruptive alteration to the DNA. Deliberately employing an endonuclease to cut a target gene can activate NHEJ which often causes a 'knock out', loss of the chosen gene's function.

Most genes occur on paired chromosomes. The other method of repair, homology-directed repair (HDR), copies and inserts the sequence from the other chromosome. When a gene that was present in only one copy is inserted onto the sister chromosome in this way, it is known as a 'homing' event (hence the 'H' in HEG). This allows the gene to spread through a population, even if it is harmful to individuals, as long as the drive advantage outweighs the fitness cost disadvantage[12]. With novel techniques using CRISPR systems, RNA-guided Cas9 can be used to cut a specific DNA sequence, and to trick the cell's repair machinery into performing HDR using a co-delivered cassette of DNA instead of the homologous chromosome[4]. This enables precise genetic manipulation, and insertion of almost any chosen DNA sequence of suitable length, resulting in gene 'knock-in' (inserting a functional gene) or in gene editing (changing the sequence of an existing gene). This provides a mechanism to drive a desirable trait through a population.

Open (intentional) release

To assess the ecological risks and potential benefits of an intentional release, it is essential to consider the invasiveness of the gene drive, in terms of both the population genetics and the population dynamics.

Some gene drive mechanisms are 'global', designed to spread throughout a population from very low initial numbers, and hence potentially to all populations, and even to closely related species. With global gene drives there may be no such thing as a confined field trial. Other 'local' gene drives can only spread if they are present above some threshold gene frequency. Threshold-based designs offer the prospect of confined trials, where effects can be studied by releasing sufficient numbers into an isolated target population, where any dispersal into non-target populations will not exceed the threshold and will not spread further. To achieve the intended benefits, larger numbers of insects must be released with local drives than global drives, impacting cost-effectiveness.

Population dynamics (e.g. spatial distribution of the organisms and how interconnected populations are) will also affect whether, how far and how quickly a gene drive spreads. We are increasingly aware that populations are genetically interconnected more than previously appreciated. Because it may be impractical to prevent gene drives spreading once released, regulations need to extend beyond those for GMOs. For example, a phased testing process recommended for trials of GM insects[25], involving step-wise increases in size and reductions in containment, cannot be adopted for gene drive systems. Mathematical modelling is valuable for informing safe regulation, as it can help regulators understand how a potential gene drive trait could spread, the impact on population genetics and, where the aim is disease control, how it affects the disease dynamics.

We recommend the following measures, which have been proposed to manage or mitigate ecological risks of gene drives [1,26]:

- Physical / barrier containment – laboratory containment and biosecurity for early-stage studies, to prevent escape into the environment;
- Ecological containment – geographical location of experimental work and testing in an inhospitable environment where escaped organisms would be unable to survive or find mates;
- Molecular containment – arrange that the drive strain is unable to have effect in susceptible populations, for example by targeting a gene that is only present in the target population (extensive monitoring would be needed to confirm this) or testing gene drive effectiveness in a laboratory population by targeting an inserted transgenic sequence so that any escapes are harmless;
- No drive – for gene editing applications separate the Cas9 and guide so that drive does not occur, and conduct field trials to investigate ecological effects using strains that are engineered to have the desired trait but without the gene drive mechanism to spread it;
- Build local rather than global drives - for example, elements which cannot spread unless they exceed a threshold frequency in the population, or multi-part genetic designs that drive initially then decay away and so have limited time to spread[27];
- Reversibility – before a modification gene drive system is released, design, build and evaluate a 'reversal drive' designed to overwrite changes spread by the first drive if required (although this would not necessarily reverse any ecological consequences suffered in the interim period).

As gene drives vary greatly, it might be useful to adopt an approach comparable to bio-containment guidelines for human or animal pathogens, where there are commonly agreed criteria that help decide what level of containment measures are needed to use in a lab environment. A similar framework for gene drive containment could help biosafety and ethics boards understand what measures would be appropriate for a particular gene drive study. With gene drives, there is a blurring of traditional lines between lab and field studies and full release, so the environmental risks should be considered at an early stage.

Resistance

As with any new technology, resistance might evolve. Resistance is not a direct ecological risk in itself. The effects of resistance to vector or pest population control are mostly economic or social – resistant populations continue to cause harm to agriculture or forestry or transmit disease. There can be indirect ecological effects, for example, reduced efficacy due to resistance tends to lead to more frequent use of chemical insecticides or herbicides and greater quantities of active ingredient. Also, when there is strong selection for resistance, other genes can be favoured by 'hitch-hiking', which might reduce genetic diversity. However, this would be true of any random mutation that provided a strong advantage in a particular environment and so became widespread.

With gene drives, the main impact of resistance will be to prevent or slow the spread of the desirable trait or population-reducing change and thus to delay or inhibit (biologically or economically) achievement of the ultimate aim. An endangered species may become extinct before a disease tolerance trait manages to spread, for example, if resistance delays it.

To enhance the chances of success of a proposed gene drive programme, the mitigation or management of resistance must be considered at an early stage. A change in DNA sequence such that the target site is not recognised and the endonuclease will not cut it, will disable the drive mechanism. Molecular designs, when selecting genes to target and the guide RNA that will recognise them, can limit such possibilities. A construct that cuts at multiple sites within one gene reduces the chance of a mutation being sufficient to negate all the cuts and thereby escape the homology-directed repair that causes the drive. However, imperfect repair by non-homologous end-joining can generate new mutations in DNA sequence; poor design could potentially result in creation and drive of resistant mutations through a population. If the desired trait is conferred by inserting co-delivered DNA, that package could become unattached from the driver, so the gene drive mechanism spreads through the population but does not achieve the desired effect. These kinds of resistance are a concern for the effectiveness, and cost-effectiveness, of gene drive technology, but are not relevant when assessing risks of detrimental ecological effects.

Where driving an inability to transmit disease into a vector population, considerations must include resistance evolving in the pathogen. Malaria parasites are sophisticated organisms with complex multi-stage life cycles, and may represent a greater risk of evolving resistance to evade pathogen-blocking or transmission-reducing traits than viruses do. There is no reason to suppose that adaptation conferring resistance to the gene drive mechanism would be any more likely to result in increased virulence or pathogenicity than any other randomly occurring mutation. Mathematical modelling could be very useful to explore the extent to which changes in pathogen traits in response to a gene drive system, and the fitness advantages (or disadvantages) of those changes, could impact on the population genetic composition and the consequences for disease dynamics.

Policy considerations

Regulations will need to be flexible to keep up with the rapid pace of technological developments. As gene drives can be so diverse – in their construction, application, objectives and environmental context – gene drives designed for release into the environment will need to be as-

Box 3. Internationally adopted standards for wooden packaging

Plant pests can take advantage of global trade to invade new areas, potentially causing economic and environmental harm. Phytosanitary (plant health) standards have been developed to prevent the international transfer of insects and diseases that could affect plants or ecosystems. The IPPC[30] operates under the auspices of the UN-FAO. Its International Standards for Phytosanitary Measures (ISPMs) are science-based and are recognised by the World Trade Organization (WTO), aiming to minimise pest risk without creating unjustified barriers to trade. ISPMs are adopted by many countries, typically through national plant protection organisations. ISPM 15 *Regulation of wood packaging material in international trade* requires wood packaging used in international trade, such as pallets, to be made from debarked wood, be heat treated or fumigated, and stamped or branded to indicate compliance. These measures are required to be applied within an official certification system and import controls are recommended to monitor compliance. ISPM 15 has been adopted by many countries, giving this international standard broad global reach and in effect harmonising regulatory requirements across much of the world.

sessed on a case-by-case basis, by species, by construct and focused on the particular receiving environment. It would be preferable to regulate the product or phenotype (the novel trait) not the process of modification[7].

When identifying potential harms and assessing their risks, regulators must judge a gene drive strategy against suitable alternatives. The appropriate comparison might be with some combination of the current control method, an idealised version of current technologies (doing what is currently done but better), or no action. It would not be appropriate to compare to an idealised risk-free alternative that does not exist in practice. For example, EU regulation of GM plants has been criticised for overzealous interpretation of the Precautionary Principle (an approach to decision-making under scientific uncertainty that is sometimes characterized as 'better safe than sorry')[28]. The whole of a proposed gene drive release programme should be considered for the comparison, not just the nature of the modified organism. This should include production of modified organisms, delivery into the environment, monitoring, concurrent use of other methods such as chemical controls, and other changes in practice such as no-till crop management. There should be consistency in matters such as what timeframe to consider in identifying potential harms and benefits, and how to apply discounting to convert monetary amounts at different time points into comparable present values.

Potential geographic spread and relative lack of reversibility are concerns for gene drive. These are also relevant to biological control, which involves the release of natural enemies to counter the impact of invasive pest animals or plants. Biological control therefore provides some precedents on which to draw, in formulating questions for regulators to consider and for suggesting experimental evidence to be provided in a permit application.

Risks should not be considered in isolation, but evaluated against some level of acceptable risk and, if not exceeding that, weighed up against the prospective benefits to decide whether it is appropriate to take those risks in order to achieve the likely benefits. Regulation of GM plants varies between regimes as to whether regulators can, or do informally, take account of benefits when performing environmental risk assessments. Risks should be viewed in the context of benefits, but formal cost-benefit analysis should not be imposed. This could be too costly, for a small organisation applicant and for a regulatory body utilising limited public funding, and would be more stringent than for other methods (chemical producers are not required to demonstrate epidemiological outcomes for vector control products). Economic and social benefits need to be more clearly articulated when thinking about risk. Public attitudes typically take account of perceived 'value' of technology, for example, opposition to GM crops is greater than that to GM vaccines.

Gene drives could potentially spread across international borders, if there is gene flow between interconnected populations, which has implications for sovereignty issues and international regulatory approval mechanisms. There is currently no real recourse in international law for such matters, except possibly by invoking trade rules if a released gene drive caused economic damage. In developing countries regulatory capacity may be lacking, understanding of the relevant science may be inadequate, and ability to make impartial assessments may be impaired. There is therefore a need to have international guidance or standards.

There are precedents around GM insects - in which gene drive research is relatively advanced - for which several guidance frameworks have been produced. The World Health Organization (WHO) issued guidance for the testing and regulation of GM mosquitoes[25], and the European Food Safety Authority published a regulatory framework for GM animals[29]. Supporting development of GM insect technologies, both of these documents recommend a tiered approach to underpin environmental risk assessment, progressing step-wise from laboratory studies (focussed on molecular biology and simple ecological processes), through contained or confined trials, to pilot implementation. At each step, scientific evaluation is accompanied by risk assessment, risk management and risk communication.

The challenge is that gene drives will be developed in a huge range of species and applications across diverse sectors – including conservation, agriculture, and human health. How could a single common statement be developed in practice that would have widespread recognition? Ideally a core set of principles could be agreed, with expansion of guidance and practical examples relating to particular fields. International co-ordination will be essential – with such a broad range of applications, no single body could have credible authority, but there are some possibilities: for example, the WHO for human health, the United Nations (UN) Food and Agriculture Organization (FAO) for agriculture, the International Plant Protection Convention (IPPC) for agricultural plant pests, the International Union for Conservation of Nature (IUCN). Such bodies should be encouraged to co-ordinate, and foster consistency.

International standards only have regulatory effect when the principles they prescribe are adopted by countries within their national legislation or requirements. However, international bodies can provide guidance to national governments, regulators or agencies, and set out standards that countries can adopt if they choose. A notable example is the widely adopted standard for wooden packaging material used to ship goods between countries (Box 3). We recommend that international standards on gene drive regulation should be developed and designed to be relevant, easy to implement, and attractive to many countries.

Conclusions

The suitability of current environmental and ecological risk assessments of gene drives is being considered, in terms of their ability to meet legitimate regulatory aims and whether requirements are achievable and desirable. Assessments must be non-discriminatory and proportionate, to protect biodiversity and minimise harm to human or animal health and the environment, whilst not being so onerous or restrictive as to quash the achievement of the potential benefits or discourage research and innovation into biotechnology solutions to significant societal or economic problems. Questions must be resolved over what information can reasonably be expected by an effective regulatory system, and how responsibility for the collection and analysis of this data should be distributed across governmental agencies, research institutions, release permit applicants and others. Such issues are under scrutiny, by policy-makers in the UK[31,32] and elsewhere. Scientists are actively involved in the debate about regulation of gene drive technologies and related societal issues[1,26].

Applied use of gene drives beyond the laboratory poses new issues that are not addressed adequately, or with sufficient clarity or scope, by existing guidance on genetically modified organisms. As gene drives blur the distinction between a field trial and implementation, and modified organisms do not respect geopolitical boundaries, an international approach is preferable for developing and maintaining guidance for regulation, and best practice for ecological risk assessments.

International bodies such as the WHO, UN and others, already have mechanisms for interacting, setting up joint working groups, for example, and recognising each other's standards in international agreements. International guidance and standards for responsible development, testing and implementation of gene drive technologies should be achievable in principle. The relevant international organisations will need both the funding and an appropriate mandate to achieve them in practice.

Recommendations

- A campaign of education and information to raise awareness among researchers and their institutions.
- An open database to provide transparent precedents for biosecurity / bioethics decisions on contained use of gene drives (where the ecological risks arise from unintentional escape), and to promote consistency across organisations and countries. Include a range of good practice examples, with an indication of the information considered and reasoning behind the decision.
- A collaboration between international bodies (such as WHO – Tropical Disease Research, UN – FAO, World Bank - Hazard Management Unit) to develop high-level ecological risk assessment principles for developing, testing and implementing gene drive technologies, and create a framework to enable development of supporting guidance relevant to particular fields in human health, animal health, agriculture and conservation.

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