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Gene Drive Organisms

Implications for the environment and nature conservation

ENVIRONMENT AGENCY AUSTRIA umweltbundesamt

GENE DRIVE ORGANISMS

Implications for the Environment and Nature Conservation

A joint technical report of the
EPA/ENCA Interest Group on
Risk Assessment and Monitoring of GMOs

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ERA
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CONTENT

EXECUTIVE SUMMARY

Biotechnology is facing profound transitions due to technological advances that augment the speed, quality and depth of genetic engineering intervention. This ever continued pace of development is posing challenges to the ability of understanding the possible impacts on biodiversity, human and animal health, environment and nature conservation. One example of a rapidly evolving scientific field in this context are genetically modified organisms (GMOs) with synthetic gene drives, also referred to as gene drive organisms (GDOs). Gene drive organisms are designed to spread genetically engineered traits into wild populations. As gene drives have been proposed to control pathogens, pests and invasive species, GDOs gain much attention in the scientific literature, the administration and the public.

GDOs represent a general shift in both, the strategy on how agricultural and environmental issues are being addressed, and how GMOs will interact with the environment. In contrast to classical GMOs, GDOs are intended to spread in the environment and will be applied to modify wildlife instead of crops. Being a powerful tool, gene drives have also been suggested to be applied in nature conservation. Because of the far-reaching consequences for the environment and nature conservation, gene drives are discussed in the EPA/ENCA Interest Group on Risk Assessment and Monitoring of GMOs.

The present document provides an overview over the technical realization of gene drives and their proposed applications, including nature conservation. The main focus of the report is on four aspects:

i) The environmental implications of GDOs

ii) The challenges that applications pose for the environmental risk assessment, monitoring and risk management

iii) Critical uncertainties associated with the approach

iv) Conceptual and legal challenges of GDO applications in nature conservation

While gene drive applications might have the potential to address environmental or human health issues, they also bear the potential for significant and irreversible environmental harm.

In order to assess gene drive applications, methods for risk assessment, environmental monitoring and risk management need to be developed and operational before any release of GDOs into the environment takes place. Due to the complexity of GDOs and its interaction with the environment, it remains unclear if and how risk assessment could result in sufficiently reliable conclusions. In parallel, societal and ethical issues need to be fully addressed when considering a GDO release.

1 BACKGROUND AND AIM OF THE REPORT

In the last years, novel techniques have become available to edit the genome of virtually any sexually reproducing organism. Some of these techniques enable the preferential inheritance of specific alleles or traits within a population, a phenomenon called `gene drive´. Being a fast evolving scientific field, many potential applications of GMOs with synthetic gene drive, also referred to as `gene drive organisms´ (GDOs), were proposed and many promises were made – including the solution of pressing challenges, such as the control or even eradication of pathogen-transmitting insect vectors, agricultural pests or invasive species.

Although most of the applications using GDOs are still conceptual and not ready for release yet, they attract much attention from the scientific literature, the media and regulators. This is mainly because the release of self-sustaining GMOs into the environment – deliberate or not – has the ability to elicit long term, large scale and potentially irreversible changes in wild populations, natural communities and even highly valued natural ecosystems. This has triggered concerns regarding appropriate provisions for the containment of these organisms and appropriate regulatory oversight and governance.

Scientific academies around the world have outlined not only the potentials, but also highlighted issues regarding the safety of gene drive applications as well as apparent gaps in the regulatory oversight (NASEM 2016, EUROPEAN COMMISSION 2017, and AAS 2017). In Europe, several countries are discussing regulatory, risk or risk assessment aspects of GDOs (AEBI & SCHOENENBERGER 2016, RIVM 2016, 2018, BIOTEKNOLOGIRADET 2017, ZKBS 2016, HCB 2017, SC NAT 2017). In June 2018, the European Commission has commissioned a mandate to the European Food Safety Authority (EFSA) for an opinion on risk assessment of gene drive organisms. The purpose of the opinion is to determine whether the existing guidance for GMO risk assessment is adequate or if updated guidance in specific areas is needed (EUROPEAN COMMISSION 2018, EFSA 2018). In addition, international bodies have addressed GDOs. The Organisation for Economic Cooperation and Development (OECD) and the World Health Organisation (WHO) have issued documents with relevance to the release of GM mosquitoes with gene drive (WHO 2014, OECD 2018). At their last meetings, the Conference of Parties to the Convention on Biological Diversity (CBD) as well as the Conference of the Parties serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety, each have adopted a decision, in which the issue of organisms containing engineered gene drives is considered (CBD 2018a, b). In both decisions, the application of the precautionary approach and the strengthening of the involvement of indigenous peoples and local communities in the decision making process when GDOs are considered for release into the environment are emphasized. Furthermore, both decisions foresee the establishment of an Ad-Hoc Technical Expert Group (AHTEG). The one under the CBD, the AHTEG on synthetic biology, at its meeting in June 2019, *inter alia* noted conceptual and legal issues that could arise by some applications of synthetic biology, including GDOs. The AHTEG on risk assessment under the Cartagena Protocol will meet in 2020 and shall *inter alia* make recommendations as to whether additional guidance on risk assessment is needed for living modified organisms containing engineered gene drives. Some of the issues addressed may warrant further consideration in cooperation with the appropriate bodies (see Chapter 8).

The relevance of gene drives has also been recognized by international conservation organisations such as the IUCN[1].

The debated risks scenarios and hypothetical benefits of GDOs have led to differing views regarding the use and release of such organisms. While some groups are in favour of a moratorium of the environmental release of GDOs due to the apparent gaps in the regulatory oversight and the potential for serious ecological and societal effects, others emphasize the potential benefits of gene drive applications and encourage further development and continued laboratory research. Meanwhile, it is apparent that decisions for the release of GDOs must not be left to risk managers alone but need a broader interdisciplinary stakeholder involvement and possibly an international governance body for the supervision and control of testing and releases of GDOs.

Against this background, the aim of this report is to delineate the potential implications of a potential use of GDOs for the environment, including nature conservation, and to address the uncertainties linked with gene drive applications. The report also analyses the challenges GDOs will pose to the environmental risk assessment, the post-release monitoring and the risk management.

[1] https://www.iucn.org/theme/science-and-economics/our-work/other-work/synthetic-biology-and-biodiversity-conservation

2 TECHNICAL REALIZATION AND CLASSIFICATION

In sexually reproducing organisms, all genomic information underlies the rules of Mendelian inheritance and evolutionary selection. However, sometimes spreading of genetic elements into populations independent of selective (dis-) advantages can be observed. Equal allele segregation can be circumvented by `greater-than-Mendelian´ or `super-Mendelian´ transmission of a specific genetic element (BURT & TRIVERS 2006). This phenomenon is referred to as gene drive. Natural gene drives are difficult to study due to the complexity and diverse mechanisms (homing endonucleases, underdominance). Even for active gene drives observed in nature, there is often uncertainty about the molecular mechanism behind the drive (e.g. MEDEA).

For a growing number of species, genetic modification has become a standard procedure in the laboratory. However, genetic modification of wild populations is hard to achieve because organisms bred in the laboratory are usually outcompeted by their wild relatives. This is even more relevant for traits that reduce the individual`s fitness. A possible solution is promised by the engineering of artificial or synthetic gene drive systems. Creating synthetic gene drives is challenging, nevertheless various strategies have already been developed (see e.g. CHAMPER et al. 2016). The general purpose of synthetic gene drives is to enable a rapid genetic modification of wild organisms by spreading a desired trait through the whole population. After the initial genetic modification and the release of few gene-drive individuals into the environment, the genetic modification step is shifted from the laboratory into the field and into natural ecosystems.

2.1 Technical realization of gene drive applications

Gene drives cannot be engineered in all types of organisms due to practical and technical restrictions. For a gene drive to spread, sexual reproduction is a prerequisite and a short generation time is highly favourable. Hence, the main targets of current gene drive research are small, often mobile animals with short generation times (e.g. insects) (see Chapter 4). With new molecular methods and tools such as CRISPR/Cas, gene drive research has become more feasible and has been accelerated. Synthetic gene drives have been shown to be functional in laboratory experiments in a number of species including yeast (DICARLO et al. 2015), fruit fly (BUCHMAN et al. 2018, GANTZ & BIER 2015a), mosquito (HAMMOND et al. 2016, GANTZ et al. 2015, WINDBICHLER et al. 2011) and recently mouse (GRUNWALD et al. 2019).

Synthetic gene drives harness a subset of different molecular mechanisms and genetic phenomena, sometimes in combination (e.g. OBERHOFER et al. 2019). The most important concepts are[2]:

1. The use of a homing endonuclease gene (HEG), which is able to cut the DNA at a desired position (BURT 2003). This can e.g. be used to copy and paste the gene drive onto the homologous chromosome, thereby converting heterozygotes to homozygotes within a single generation. Another mechanism uses HEGs to cleave an essential gene, while at the same time providing a rescued version of that gene (OBERHOFER et al. 2019). HEGs still remain difficult to engineer in the laboratory. With the exploitation of CRISPR/Cas, which can easily be engineered to function as a HEG, gene drive research was considerably enhanced (ESVELT et al. 2014).
2. Genetic material which hinders inheritance can favour and drive certain alleles within a population. Naturally occurring gene drives with a biased production of gametes has for example been shown in fruit fly (segregation distorter), flour beetle (MEDEA) or the house mouse (t-haplotype). Such gene drives can be engineered in the laboratory using different approaches, e.g. chromosomal rearrangements or toxin/antidote systems (CHEN et al. 2007, AKBARI et al. 2013).

2.2 Classification of gene drives

Gene drives can be designed to introduce and spread a genetic modification permanently into a whole population or species. Those gene drives are referred to as modification drives. The introduced gene changes a desired trait in all individuals of a natural population. Those gene drives and the introduced modification can be designed to persist in the target population unlimited in time and space, resulting in a replacement of the natural population.

A second strategy introduces gene drives to reduce or even eliminate a population or species. Those gene drives are referred to as suppression drives. The latter result in a fitness reduction in the overall population or species, eventually causing it to crash. While a successful eradication of the target population will also remove the gene drive, including the genetic modification, from the environment, the consequences of the gene drive will remain.

The mechanism of the specific gene-drive application determines its propagation behaviour. Gene drives can be categorized as global, when they potentially affect all organisms of a species, or local, when they aim to affect only a determined (sub-)population. Different mechanisms were theoretically proposed and practically used in laboratory experiments to engineer those propagation behaviours. A key property of each gene-drive concept is its ability or inability to be confined to certain individuals or populations. Intrinsically, local drives involve a trade-off: In order to remain local, gene drive organisms need to be released at

[2] Not considered here is the use of microorganisms (*Wolbachia*) to skew inheritance rates of insects (mosquitoes), as biotechnology is solely used to introduce the microorganism into the insect and not to manipulate the organisms themselves (microorganism or the insect).

a high initial threshold. The threshold needed for the drive to take effect depends on the mechanism and the molecular construction of the drive as well as on the characteristics of the target population. Thresholds typically vary between 25% and 60% of organisms that need to carry a gene drive per population. In the case of the eradication of invasive rodents on islands (see Chapter 4), the implementation of the drive would have to be linked to a massive release of gene drive rodents. Despite this drawback, threshold-dependent gene drive systems theoretically offer the advantage that the gene drive is less likely to spread between two populations if the migration between those populations is lower than the threshold.

Low or no threshold gene drives may become global by being able to spread to whole populations and migrate between populations (e.g. global `CRISPR´ drive). Intrinsically, a spatial control of threshold independent gene drives is not possible on the molecular level. Concepts to limit the spread of the gene drive in time (number of generations) were proposed but have not yet been shown in practice (e.g. NOBLE et al. 2016). Global gene drives have the potential to affect all individuals of a given species (or species complex). Conceptually, such gene drives offer the option to either create an entirely genetically modified species (global modification drive) or to induce global extinction (global suppression drive).

The possibility to reverse a gene drive is in practice of great importance because of the potentially high impact GDOs may have on ecosystems and because of the capacity of some gene drives to theoretically spread unlimited in time and space (intended or unintended). Concepts for reversibility of gene drives have been theoretically proposed for many gene-drive systems and modelled in some cases. Mechanistically, reversal can be achieved either by `flooding´ the population with wild type individuals or the release of additional, counteracting drives. Both approaches contain significant uncertainties and limitations with possible negative effects on the environment. As synthetic gene drives have not been tested in the field so far, no data are available that can demonstrate the feasibility of the proposed concepts to reverse gene drives.

3 GENE DRIVE APPLICATIONS – AN OVERVIEW

Since the first proof-of-concept studies have shown the functionality of gene drives under laboratory conditions (e.g. WINDBICHLER et al. 2011, AKBARI et al. 2013, GANTZ & BIER 2015a, 2015b, HAMMOND et al. 2017), this new technique was praised as powerful, precise and promising remedy for a range of globally or locally relevant environmental or human health problems (ESVELT et al. 2014, NASEM 2016, AAS 2017). Despite their novelty and complexity, GDOs may be ready for environmental release within the next decade. First releases of GDOs were projected for mid-2020 (BARTSCH et al. 2017) or even earlier (SCUDELLARI 2019).

Probably the most widely discussed example of a gene drive application aims to deploy gene drive mosquitoes in order to substantially reduce populations of malaria-transmitting mosquitoes in sub-Saharan Africa (NAJJAR et al. 2017). This project is promoted by the research alliance 'Target Malaria' and supported by the Bill & Melinda Gates Foundation[3]. By applying a phased approach, genetically modified mosquitoes without gene drive would be tested first. Considering the commitment of African leadership to eliminate malaria by 2030[4], the current ambition of the Target Malaria alliance to conduct first field releases of gene-drive organisms in 2026[5] seems to be realistic. Another example of research motivated to solve health issues is the `mice against ticks´ project, which aims at immunizing the white-footed mice on Nantucket Island, USA, in order to deplete one specific vector reservoir of the Lyme pathogen (NAJJAR et al. 2017). Although currently cisgenic mice are being developed, the use of a local gene drive approach has also been proposed as an option to spread immunized mice across the island (BUCHTHAL et al. 2018).

Gene drive technology has also been proposed to be used in agricultural breeding, e.g. as a tool to speed up the breeding process of livestock (GONEN et al. 2017). Another target of the gene drive technology is the control of weeds, pests or pathogens in agriculture (NASEM 2016). In the USA, agricultural producer representatives have already expressed commercial interest in gene drive applications targeting agricultural pests and have co-funded related research programs (ETC 2018). Experiments have shown the functionality of a gene drive in the spotted wing fruit flies (Diptera: *Drosophila suzukii*), an invasive pest from Japan damaging small fruit crops (BUCHMAN et al. 2017). Other target pests are the Mediterreanean fruit fly (Diptera: *Ceratitis capitata*), moths like the cotton leafworm (Lepidoptera: *Spodoptera littoralis*), aphids, plant hoppers, whiteflies or nematodes (ETC 2018, SCOTT et al. 2018). A different field of gene drive research aims to develop gene drives to restore herbicide susceptibility in resistant weedy plant species, e.g. horseweed or pigweed (ESVELT et al. 2014, OYE et al. 2014, NASEM 2016, MIN et al. 2018). By this approach, genetic adaptations in weed populations that provide resistance to herbicides could be reversed (re-sensitizing drives). Sensitizing drives could also confer vulnerability

[3] https://targetmalaria.org/.

[4] http://alma2030org.content/african-heads-state-adopt-roadmap-eliminate-malaria-afrika-2030

[5] https://www.economist.com/briefing/2018/11/08/the-promise-and-peril-of-gene-drives and https://www.businessinsider.de/target-malaria-wants-to-end-mosquito-borne-disease-using-gene-drives-2019-1?r=US&IR=T

of a (hitherto non-resistant) weed species to a particular new chemical compound to be used as novel control strategy. Another approach targets sex-specific genes for weed population suppression. However, several technical challenges still have to be solved before gene drives can be applied in plants.

Another, still hypothetical, use of gene drive technology targets pest behaviour or ecological services in agriculture (ETC 2018). A US company has filed a patent aiming at inserting a switchable optogenetic gene into honey bees by use of gene drive, thereby allowing farmers to attract the beneficial insects for pollination using an external light source. Alternatively, it has been suggested to deter pest or noxious insects from humans, crops and livestock by changing their behaviour. Another proposal for agricultural benefit included the release of GDOs to remove genetic pollution caused by GMOs (ETC 2018). Specifically designed GDOs could interbreed with GMOs in the wild, thereby restoring the wild type variety.

The US Defence Advanced Research Projects Agency (DARPA) supports research aiming at the safety and accuracy of genome editing and gene drives[6] (RUDENKO et al. 2018). These activities triggered concerns about the potential dual use of gene drive applications, in particular regarding potential military use[7] (ETC 2018). These concerns are further pushed by reports on research by DARPA on non gene drive-based agricultural genetic technologies aiming at horizontal environmental genetic alteration agents, such as the dispersal of infectious GM viruses to edit crop chromosomes *in situ* (REEVES et al. 2018).

The potentially beneficial use of gene drive technology for conservation purposes has also been proposed and is dealt with in Chapter 5.

[6] https://www.darpa.mil/program/safe-genes

[7] https://www.theguardian.com/science/2017/dec/04/us-military-agency-invests-100m-in-genetic-extinction-technologies

4 GENE DRIVE APPROACHES FOR NATURE CONSERVATION

As gene-drive applications offer the possibility to modify wild populations, it is not surprising that some concepts using GDOs aim at benefiting nature conservation. In fact, an increasing number of publications promote the use of gene drive to achieve protection goals linked to the conservation of species and ecosystems (e.g. JOHNSON et al. 2016, PIAGGIO et al. 2017, RODE et al. 2018, PHELPS et al. 2019, REDFORD et al. 2019).

The use of GDOs is only one example of synthetic biology targeting nature conservation. Other projects relate to the restoration of species (de-extinction projects), or aim to ease the pressure on wildlife trade with endangered species (e.g. synthetic rhino horn or alternatives for the amoebocyte lysate from horseshoe crabs). The latter also relate to conservation issues but are out of the scope of this document.

Despite the large body of publications that emerges in the field of nature conservation and synthetic biology, the present approaches to use gene drive in nature conservation focus on only few applications. Most of them are related to the control or the eradication of invasive species (e.g. suppression drives). Few are concerned with the possibility to drive alleles into natural populations to increase their resilience to stressors such as pathogens (e.g. modification drives).

Given that conventional means to reduce invasive species are non-specific (e.g. poisons) and labour intensive, controlling invasive species with gene drive is believed to save costs and to be environmentally more benign (CAMPBELL et al. 2015, LEITSCHUH et al. 2018), especially when applied in larger areas (HARVEY-SAMUEL et al. 2017). Although most approaches to control invasive species target economic pests, there is a sliding transition from pest management to nature conservation, as some economic pests may also threaten biodiversity and protected species. Some researchers therefore propagate gene drive as a means to control (invasive) species harmful for biodiversity. Recent publications exemplify the broad range of species which may be targeted by gene drive (NASEM 2016, MORO et al. 2018, DEARDEN et al. 2018). Examples include many taxa such as starfish, wasps, toads, snakes, possums, rats, foxes or starlings. Especially in Australia and New Zealand, research Institutions such as CSIRO analyse whether gene drive approaches can be used as a tool to eliminate invasive fauna and flora, mainly introduced from Europe[8].

Because confinement, both in space and time, will be a key issue for the practical implementation of the first GDOs released, invasive species on islands are the most likely candidates for a gene drive application to be put in practice. A prominent example is the `genetic biocontrol of invasive rodents´[9] supported by conservation organizations[10], governmental bodies (USDA, CSIRO) as well as universities (e.g. North Caroline State University). One of the affiliated groups at the US North Caroline State University is currently developing gene drive mice

[8] https://www.csiro.au/

[9] https://www.geneticbiocontrol.org/

[10] https://www.islandconservation.org/

using the `daughterless-gene approach´. The gene drive is meant to decrease invasive mouse populations by use of a meiotic gene drive system, making all offspring either male or sterile. The conservation goal in this context is to relieve the pressure caused by rodents on threatened bird populations.

Another gene drive application linked to the protection of bird populations has a focus on avian malaria. In this project, negative effects of the pathogen on the bird population of Hawaii are to be countered by suppressing the mosquito vector, which is also invasive on Hawaii. In this case, researchers justify the use of GDOs by stating that current conservation strategies may be insufficient to protect bird populations, especially at higher elevations (LIAO et al. 2017). Other ideas aim to manipulate protected species by enhancing their fitness and resilience to stressors. To do so, gene drives could deliver the respective genomic changes into wild populations. Examples of such research are considerations to combat the bacterial infection of amphibians with chytrid fungi which are a worldwide serious threat to amphibians. Other ideas aim to modify dinoflagellates which are associated with corals to lessen the effect of global warming on coral bleaching.

Apart from projects using gene-drive applications with a clear focus on nature conservation, a range of gene drive applications may impact nature conservation goals indirectly. Using gene drives as a means to assist pest management means that wild populations may be modified, reduced or eradicated in a way and on a scale which is unprecedented. The same argument holds true for the most prominent project involving gene drive, the `Target Malaria Project´ (see Chapter 4). Reduction of population size or eradication of a population or species will have consequences for food webs and ecosystems that are difficult to assess (see Chapter 6). It is therefore important to stress that gene drive technology has the potential to affect nature conservation in both, potentially positive and negative, ways. This is particularly paradoxical in the case of projects with the intention to embrace GDOs to counter invasive species. On the one hand, their disappearance from the ecosystem they invaded might substantially benefit the local conservation efforts. On the other hand, they have a large potential to cause environmental harm if GDOs escape to the geographic regions where these species are native or have their centre of origin. Any escape of GDOs from the intended localities into such regions could elicit severe negative consequences for nature conservation.

5 ENVIRONMENTAL IMPLICATIONS

5.1 Potential risks of gene drive organisms for the environment

Organisms with gene drives represent a fundamental shift in the way how public health, agricultural and environmental issues are being addressed and how genetically modified organisms will interact with the environment., In contrast to classical GMOs, where the spread into the environment and the diffusion of the genetic modification into wild populations is not intentional, GDO releases are intended for modification of wild populations. Thereby, GDOs essentially induce the modification of natural populations after their release into the environment. By doing so, wild species are genetically modified and exploited to further spread the genetic construct by inheritance. In addition, up to now crop plants were protected against pests and pathogens by the use of resistant varieties, plant protection agents or specific management methods. When applying GDOs, protective measures are typically intended to affect individuals of the noxious species also outside the cultivation area of a specific crop plant. In this regard GDOs represent a fundamental strategy change when compared to environmental releases of GMOs (SIMON et al. 2018).

Gene flow between a GM plant and a wild or weedy relative has so far been considered an unintended consequence which may result in adverse environmental effects (EFSA 2010, ELLSTRAND et al. 2013). In contrast, with GDOs the highly accelerated and possibly total and infinite spread of edited genes or novel traits within populations typically is a key feature of the gene drive technology with profound implications for the targeted wild population as well as the ecosystems in which the organisms are released.

Another important issue is the likelihood of gene flow and hybridisation between different species or subspecies. If interspecific gene flow and hybridization of wild species with a gene drive with related species is theoretically possible, then effects on species other than the target species need to be considered (particularly if the gene drive target site or a similar sequence is present). For example, gene drive applications have been proposed to suppress or even eradicate disease-transmitting mosquito species (Chapter 4). Interspecific gene flow and hybridization between different mosquito species within the genus *Anopheles* and the potential for adaptive gene flow within this genus across the African continent has been highlighted (MILES et al. 2016). Thus, suppression drives have the potential to eradicate species, also other than the target one.

Suppression drives could also impact food chains and whole ecosystems and their biodiversity, e.g. by the loss of prey and important food sources for higher trophic levels. Depending on the specific role of the species targeted by gene drive technology in a particular ecosystem, predators, prey species, competitors or even complex ecological functions may be negatively affected.

Releasing GDOs may also have evolutionary consequences that are presently poorly understood. The introduction of novel mortality factors or selection pressures in a wild population may even have evolutionary consequences on non-target organisms. The reduction of genetic diversity in the remaining population, changes in vector biology or in ecological interactions are likely (DAVID et al. 2013). Depending on the specific gene drive mechanism applied and the gene

drive`s ability to spread within a population, the evolutionary consequences for the targeted population comprise decreased fitness, population declines and risk of extinction for a (meta)population or the whole species. Experience with (insect) species released for the purpose of classical biological pest control has shown that species used for biocontrol can become invasive, particularly in non-native environments, with diverse ramifications for the non-target species and the environment (LOUDA et al. 2003). CRISPR-based gene drives are likely to be invasive in wild populations with unknown potential for ecological effects (NOBLE et al. 2018). Other effects may include depletion of the genetic or phenotypic diversity and changes in the mating system of a species (DAVID et al. 2013, BULL 2016). For gene drive applications targeting pathogen-transmitting vectors such as mosquitoes, evolutionary responses of the pathogens due to changes in its vector population are considered possible (MEDLOCK et al. 2009).

A prominently discussed effect is the loss of functionality and durability of the gene drive due to the occurrence of resistance to the gene drive mechanism in the target population (see also 7.1). Failures of the cell`s repair mechanism during the cut and repair process of the DNA, but also mutations in the genome of the DNA or of any enzymatic component of the drive mechanism may lead to molecular resistance to the gene drive (CHAMPER et al. 2017). Behavioural resistance can induce changes in mating, feeding or breeding behaviour in the wild population, thereby circumventing the spread of the gene drive (MCINNIS et al. 1996, BULL 2015). The occurrence of molecular resistance has not only been theoretically explored but also shown in cage experiments and represents a major obstacle in the practical deployment of GDOs (CALLAWAY 2017a, HAMMOND et al. 2017). In case resistance occurs, the effectiveness of the gene drive mechanism is not provided, and the aim of the gene drive application will not be achieved. Although a lack of function of a gene drive may not be considered *per se* as an ecological risk, it may entail risks to human health and the environment, e.g. in particular if conventional control strategies have been changed or halted (MURPHY et al. 2010, DAVID et al. 2013, BULL 2015). A key question in this regard is how long a gene drive needs to remain functional in order to achieve the goal of its release and if this can be reliably predicted.

5.2 Challenges for the environmental risk assessment

As GDOs are genetically modified organisms, they will be subject to existing GMO regulation in the European Union, with risk assessment and post-release monitoring. Environmental risk assessment (ERA) is a structured process with the aim to identify risks of a particular stressor to specific protection goals and to estimate the probability with which anticipated adverse effects are likely to occur. In the EU, as in many other countries, the ERA is science-based and on a case-by-case basis. Several guidance documents for the ERA of GMOs are available, such as for GM plants, GM animals including GM insects as well as for specific aspects of the ERA (e.g. EFSA 2010, 2011a and 2013). Specifically for the ERA of GM insects, guidelines have been published by the WHO (WHO 2014). However, as most GMOs today are crop plants, the available guidelines and experiences do neither reflect the aforementioned fundamental shift from GMOs to GDOs, nor the shift from crop plants to wild species.

Compared to classical GMOs, the potential adverse effects of GDOs will certainly differ in magnitude and permanence due to the large scale and long-term character of the gene drive applications proposed so far. Hence, the quantification of risks to the environment by the application of GDOs may be very difficult. The inherent characteristics of GDOs - the intentional and long-term, potentially unlimited, spread and persistence of novel genes and traits in wild populations – may account for high uncertainty in predictions of the adverse effects and speculative risk estimations (Chapter 7). Delayed and large-scale risks pose new challenges to the risk assessment as appropriate methodologies are currently lacking. Also, in many cases the necessary data on the biology, ecology and population dynamics of target and non-target species will be missing.

Consequently, risk assessment methodologies need to be adapted to the specificities of GDOs with more emphasis put on the assessment of uncertainties and recognition of knowledge gaps. In principle, current ERA provisions in the EU allow the identification and estimation of uncertainties and knowledge gaps (EFSA SCIENTIFIC COMMITTEE 2018). However, in both the EU and in the US, the adequate consideration of uncertainties in the ERA of GM crops and GM mosquitoes has been questioned (HILBECK et al. 2011, MEGHANI & KUZMA 2018).

Another issue which needs to be addressed is the step-by-step principle or stepwise approach foreseen for the release of GMO in Europe (EUROPEAN COMMISSION 2002, EFSA 2010). The principle ensures a phased testing regime, starting with laboratory tests and some form of physical or ecological confinement before conducting open field trials and environmental releases. The rationale behind this approach is that a GMO shall be gradually released into the environment, only if its safe use at the previous step can be shown. For GDOs, such a gradual reduction of containment and increase in environmental release is, in practice, difficult to achieve, as a single release of a GDO into the environment may spread into the whole population (NOBLE et al. 2018, SIMON et al. 2018). As the outcome of a GDO escape may pose high risks, more research is needed on how to experimentally test GDOs in a contained environment, both in space and in time. First proposals for confinement methods for lab experiments and field trials (molecular, ecological, and physical) have been made (AKBARI et al. 2015, RIVM 2017) but not yet applied. In addition, available risk mitigation measures are a necessary requirement before first field releases can be conducted (OYE et al 2014).

National GMO regulations and ERA provisions will not suffice to cover the specific challenges of GDOs for the assessment of environmental and human health risks. The potential spread and transboundary movement of GDOs across national borders and their potential impact on different communities poses novel governance issues, which cannot be covered by current ERA provisions. Ethical and societal considerations need to be addressed even before GDOs are being developed (Kofler et al. 2018). Last but not least, the question of the retrievability of a GDO has to be addressed. For classical GMOs, the approval of the release into the environment has to be renewed when the consent period (usually 10 years) has expired. In case no re-approval is granted, placing on the market and therefore further environmental release is not allowed. For classical, non-spreading GMOs this is feasible, while the retrieval of continuously spreading GDOs is hardly possible once release into the environment has occurred.

In the European Union, the existing regulatory provisions for GMOs such as Directive 2001/18/EC and its Annexes, Commission Directive (EU) 2018/350, as well as the Guidance Documents issued by EFSA will be useful as a starting point to carry out the ERA also for GDOs. However, the potential shortcomings of currently applied risk assessment requirements for GDOs have to be recognized and a review of current risk assessment provisions with regard to their eligibility for GDOs is envisaged until 2020 (EUROPEAN COMMISSION 2018). Also outside the EU, controversies about the regulation of GD applications emerge and challenges for risk assessment requirements also in other countries have been highlighted (OYE et al 2014, NASEM 2016, AAS 2017, CALLAWAY 2017b, ECNH 2018). Experience and regulations from other areas, such as the assessment of biocontrol agents or efforts to control invasive species also can provide valuable information.

5.3 Challenges for the environmental monitoring

The monitoring of environmental effects after release of a GMO into the environment is an important element of the regulatory framework for GMOs in the EU. Experimental releases as well as the placing on the market of GMOs have to be accompanied by monitoring measures with the aim to detect effects of the GMO to human health and the environment, and to facilitate – where required – early and appropriate mitigation action.

The design of each post-market monitoring should be science-based and closely related to the outcome of the case-by-case ERA. A guidance document for post-market environmental monitoring (PMEM) is so far only available for GM plants (EFSA 2011b). Several additional publications contribute to the implementation of an adequate EU-wide PMEM standard of GM plants (e.g. PASCHER et al. 2010, UMWELTBUNDESAMT 2011, ZÜGHART et al. 2013). Similar to ERA guidance, all these documents neither reflect the specific challenges of monitoring (effects of) GDOs nor those of wild species.

The current PMEM provisions therefore need to be adapted, further developed or newly designed to meet the specific requirements of GDOs. Particularly the ability of GDOs to induce long term, large scale and potentially irreversible changes in wild populations, natural communities and natural ecosystems calls for adequate methodologies, monitoring locations and monitoring time frames. The observation and documentation of the exposure of GDOs to the environment is an important basis for the detection of harmful effects and the derivation of cause-effect relationships. Therefore surveying the efficacy of the GDOs as well as their presence and prevalence in wild populations are important tasks of the PMEM. In addition, PMEM must ensure that potential adverse effects on the environment are timely detected, in particular if these become only evident in large-scale applications (DEVOS et al. 2016). Because of the novel features of GDOs and the potential for long-term environmental harm, sufficient funding for a long-term PMEM has to be provided.

For GDOs as a public health tool, particularly the epidemiological impact as well as potential side effects of the application must be constantly monitored, similarly to pharmacovigilance of medicinal products (WHO 2014). In this context, criteria for the acceptability of risks for effects of GDOs on human health and the environment will be inevitable in order to facilitate decision-making before releasing GDOs into the environment.

6 CRITICAL UNCERTAINTIES OF GENE DRIVE ORGANISMS

So far, gene drive applications are mostly theoretical concepts although few applications are in preparation for field releases. Evidence for the functionality of GDOs is provided largely by proof-of-concept studies with selected organisms in the laboratory while operational use of GDOs under realistic environmental conditions is still lacking. Hence, several uncertainties regarding GDOs have to be highlighted.

6.1 Uncertainty of the evolutionary robustness of the gene drive mechanism in the environment

The functionality of a specific gene drive approach under realistic environmental conditions is one of the major question marks framing the discussion on gene drives and their promises to solve some of the most pressing environmental and human health issues. The efficacy of gene drive mechanisms has so far only been demonstrated in laboratory or cage experiments, predominantly with unsatisfactory results (e.g. GRUNWALD et al. 2019). Depending on the targeted species and the specific drive approach chosen, the synthetic trait has been spread throughout a target population after 3 to 20 generations under controlled conditions (GANTZ et al. 2015, HAMMOND et al. 2017, BUCHMAN et al. 2018).

However, GDOs used in experiments so far are usually genetically uniform strains, which can only be bred in the laboratory. Such strains cannot be fully compared to wild populations. Wild population are genetically more diverse, with different genetic backgrounds and variations in their target sequences, as shown for the Malaria-transmitting species *Anopheles gambiae* (MILES et al. 2016). Such genetic variability can lower the conversion efficiency of synthetic CRISPR gene drives and therefore hamper the spread of the intended trait (DRURY et al. 2016, CHAMPER et al. 2017). In addition, differences in ecological factors between wild-type organisms and GDOs with regard to dispersal or mating behaviour can affect the success of a gene drive (ECKHOFF et al. 2016). Also sub-optimal conditions during environmental release with regard to food availability, the presence of competitors, predators, mating conditions and climatic factors or temporal and spatial connectivity of sub-populations can affect the transmission rate of the edited gene and consequently the effectiveness of the GD *in situ*.

A major constraint of currently developed gene drives is the fast evolution of resistance to the drive, which may hamper the spread of the drive in a population. Resistance to the drive occurs at the molecular level if the targeted sequence mutates, either naturally or due to errors in the cell`s DNA repair system during the copying process, or because of interferences with the CRISPR mechanism at RNA or protein level (ESVELT et al. 2014, BULL & MALIK 2017). Because of the high selection pressure, such mutations typically spread fast in the target population. Alternative gene drive architectures, e.g. by targeting multiple genes, several sites within the gene or targeting highly conserved genes, have been proposed in order to avoid resistance development (e.g. NOBLE et al. 2017,

CHAMPER et al. 2018). Another approach is targeting one or multiple sites in highly conserved vital genes, where mutations are less likely and, if they occur, very probably lethal to the individual (KYROU et al. 2018). However, these approaches are still mainly theoretical and also require scientific evidence of their functionality under realistic conditions. Another possibility for evolutionary escape of target organisms from gene drives is based on the behavioural plasticity of organisms, specifically through altered or non-random mating behaviour of the targeted species (MCINNIS et al. 1996, DRURY et al. 2016, ZENTNER & WADE 2017). Evolutionary decay of the gene drive can also be mediated by vector or parasite resistance to the drive (BULL 2015). Several pathways for GDOs to develop resistance to the GD and to overcome the evolutionary stability of a gene drive have been theoretically identified (CHAMPER et al. 2017). However, predicting the different escape mechanisms to a specific gene drive approach can only be achieved through experience (BULL 2015). While failure rates of conventional eradication techniques of invasive species due to resistance are well known, these are also likely but highly unpredictable for gene drive-based control methods (LEITSCHUH et al. 2018).

6.2 Knowledge gaps in the biology and ecology of wild species

Gene drive technology allows targeting a broad range of wild organisms from yeast to mammals. So far, GMOs released into the environment, were mostly crop species with a long history of use in agriculture, a limited genetic variability as well as extensive knowledge of their (molecular) biology and environmental behaviour in a human-controlled agro-environment as the basis for a science-based evaluation of potential risks (Chapter 6). In contrast, our knowledge on the biology, ecology, genome-environment interactions, ecosystem role and function of wild species in natural environments is incomplete, as it is for general ecosystem functioning and community dynamics. For example in the case of gene drive applications targeting weed species, polygenic resistance mechanisms are not yet fully understood (NEVE 2018). For mosquitoes, as potential first target organisms for gene drive, their long-distance dispersal ability high above ground has been discovered only recently, contrasting previous predictions (LEHMANN et al. 2018). Experience with pest eradication programs such as the New World screwworm fly has shown that knowledge on the ecological impact of pest species as well as of their eradication is limited and more ecological knowledge is required before and after population suppression programs (SCOTT et al. 2018). For the ERA, the modelling of large-scale or long-term effects requires sound scientific data on population and ecological parameters rather than vague assumptions. Hence, uncertainties and knowledge gaps of basic biological parameters may result in highly speculative risk estimations.

6.3 Unintended effects at the molecular level with unknown ramifications for gene-environment interactions

The design of a synthetic gene drive is associated with components that are novel (e.g. CRISPR/Cas in eukaryotes) and complex (e.g. toxin/antidote systems based on RNAi), all of which need to be thoroughly concerted (Grunwald et al. 2019). In this context, target specificity of site-specific nucleases is important and genome editing at off-target sites has been reported for many of the approaches used for GDOs as unintended effects at the molecular level (see summary in UMWELTBUNDESAMT 2014). Once CRISPR-GDOs have been released, the specific gene drive mechanism will repeatedly be copied into each new generation. For homing endonucleases, a longstanding history of safe use, e.g. regarding eukaryotic genome stability, is lacking. This is true for both, the mechanistic components that ensure super-Mendelian inheritance as well as the payload gene, taken from a different species, inducing the functional change. In the long term, off-target effects and unintended effects at the molecular level may occur with unknown ramifications for the resulting phenotype as well as for genome-environment interactions. Gene drive mechanisms act by skewing natural inheritance. In order to achieve this, its molecular components are often acting in the germline, at pivotal and vulnerable stages of development (e.g. during spermatogenesis, gametogenesis) or in early zygotes. Viable deviations of the intended modification happening in the germline will result in inheritance of the undesired change. With gene drives, novel complex and synthetic molecular components are released into the environment and might remain there indefinitely.

6.4 Lack of reliable containment of and reversal methods for gene drive organisms

One of the major concerns of GDOs is their accidental release from containments and consequent unintended spread in target and non-target populations. Hence, strategies and safeguards have been suggested to prevent such uncontrolled releases of GDOs, either at the molecular level or by physical or ecological containment, or by a combination of these (AKBARI et al. 2015). Physical and ecological containment methods aim at restricting GDOs to enclosed environments or to geographical locations where no wild populations of the target organism exist. While there is experience with the physical containment of GM insects as well as respective guidelines for its implementation (BENEDICT et al. 2008, WHO 2014), ecological confinement can be easily overcome if assumptions on ecological conditions turn out to be erroneous, e.g. regarding the dispersal ability of the target organisms or if accidental or deliberate transport of GDOs into their native environments occurs. Therefore, additional molecular mitigation strategies have been called for in case GDOs spread into non-target populations or if other adverse effects become evident. For threshold dependent gene drives, the release of wild-type organisms was proposed to dilute a gene drive-modified target population, thereby phasing out the gene drive (AKBARI et al. 2013). For homing endonucleases such as CRISPR/Cas gene drives, molecular countermeasures for their reversal have been explored that are also based on gene drive mechanisms (ESVELT et al. 2014). These aim at

deleting or replacing the original gene drive or rendering wild-type organisms immune or resistant to the original drive (GANTZ & BIER 2015b, ESVELT et al. 2014, VELLA et al. 2017, MARSHALL & AKBARI 2017). Predictions on the efficacy of these methods are theoretical and controversial as the success of some of the proposed methods varies with the underlying assumptions (VELLA et al. 2017). Because the counter gene drive requires time to pervade the population, the negative environmental effects by the original gene drive cannot be stopped immediately. Even if the original gene drive can finally be halted and reversed, a full restoration of the wild type genotype cannot be achieved due to the presence of certain synthetic molecular elements in the target population. Hence the proposed remediation strategies for unwanted gene drives are still theoretical and require scientific evidence of their functionality under realistic conditions.

7 A BROADER TECHNOLOGY ASSESSMENT PERSPECTIVE

7.1 Legal and conceptual challenges of GDO applications in nature conservation

Genetic-engineering applications including GDOs in nature conservation pose conceptual and legal challenges for nature conservation, which add to the highly complex risk assessment[11]. While challenges for the risk assessment of GDOs are currently discussed on different levels, conceptual and legal challenges are hardly considered at present.

Legal challenges concern the status of originally protected species which have been genetically modified by genetic engineering. It needs to be clarified for example, if these GMOs keep their status as protected species and – when applicable – their status in CITES[12], as they are not a wildtype organism of the species to which the protection status has been granted. Also, the status of the GMO as a potentially invasive species will need to be evaluated, since the genetic modification of wild populations could be classified as non-native in their native range.

In addition, questions on a conceptual level need to be addressed with great care. These include the legitimacy and the basic impact of genetic engineering as a tool in nature conservation. For example, the relation between naturalness and artificiality needs to be evaluated with respect to nature-conservation concepts. The further genetic engineering proceeds to create artificial organisms, the greater will be the influence on concepts and ideas of life and nature. The discussion as to whether genetic engineering is permissible in nature conservation must therefore also focus on the search for a borderline between the evolved and the increasing mechanisation of organisms and ecosystems. The question arises as to whether the use of genetic engineering and in particular GDOs in nature conservation is a critical transgression of this borderline. It has also to be discussed whether genetic modifications of ecosystems are conceptually compatible with our to-date definitions and understanding of biological diversity.

Challenges on a more concrete level include applications in which protected species are intended to be made resistant to disease by genetic engineering, because natural resistances are not likely to evolve any more. Such applications reflect a rather static understanding of nature conservation, which has to be taken into account.

The evaluation of the appropriateness of the use of genetic engineering as a tool in nature conservation also needs to include a comparison with other potential tools. What are the risks of a GMO-oriented approach to nature conservation that combats the problems symptomatically rather than remedying the causes? To what extent does the use of genetic engineering compete on a resource level with other means and instruments of nature conservation, and how should the use of resources be weighted? In the context of AHTEG Synthetic

[11] https://www.cbd.int/meetings/SYNBIO-AHTEG-2019-01, page 9

[12] https://www.cites.org/

Biology, the effects of genetic engineering applications in nature conservation on the cultural practices of Indigenous People and Local Communities (IPLCs) were discussed. The focus in the discussion was on the potential impact of GDO on the cultural practice of IPLCs to maintain the balance of nature's elements and to their ability to live in harmony with Mother Nature.

In summary, there are challenges to GDOs application in nature conservation that need further investigations and evaluations, both on a scientific level and on a societal normative level. For such an evaluation, instruments of technology assessment might be helpful and warrant a structure in the discourse.

7.2 A technology assessment perspective

A technology assessment approach could provide instruments to evaluate GDOs on a broader level. Technology assessment is an interdisciplinary scientific approach that has developed scientific methods and comprehensible criteria to assess technical applications and to propose possible courses of action. At a fundamental level, this approach can discuss the prerequisites, consequences and appropriateness of the technology compared to other means for achieving environmental and societal goals. On a larger scale, it could include an assessment of the social, economic and cultural impacts that go beyond the risk assessment of GDOs. Technology assessment can help to create a better information base and provide normative orientation. Even if technology assessment tools are currently available on a general level to evaluate genetic engineering, the technology assessment concepts and methods will need to be substantiated and driven forward before being applied to gene drive applications in the context of nature protection.

8 CONCLUSIONS

Novel genetic approaches are in the phase of development to address environmental, agricultural and public health issues. Synthetic gene drives represent one of these approaches and have the power to spread genes, including genes with negative fitness effects, through a natural population or species.

Although the main focus of gene drive research is on human health (i.e. control of vector borne diseases) or the management of pests and invasive species, applications specifically designed for nature conservation are also discussed. Of all applications for nature conservation proposed so far, the use of suppression drives to eliminate invasive species on islands have the highest likelihood for realization. However, all gene drive applications are at a very early stage of research and must overcome many technical and practical problems before being available for release into the environment.

Because gene drives have the potential to wipe out populations over large areas or even affect whole species and ecosystems, their ecological implications and environmental risks are likely to be considerable. The present report highlights these risks as well as the critical uncertainties and knowledge gaps, both in the technology itself and in the data and methods available for the environmental risk assessment and monitoring. Due to the long term character of gene drive applications, the consideration of these knowledge gaps and uncertainties is of critical importance to assess the technology.

GDOs differ in many ways from classical GMOs. The potential of these novel genetic approaches to genetically modify or eradicate wild populations is as yet unprecedented. The release of GDOs has the ability for an unparalleled temporal and spatial exposure of the environment and severe implications for nature conservation goals. It is therefore crucial to scrutinize and update the environmental risk assessment and the post-release monitoring in order to be fit for purpose *before* first environmental releases of GDOs are performed, if gene drives are to be considered a viable option in the future. Today`s risk assessment tools are not ready and the required knowledge and data are not available to assess the highly complex risk scenarios and relationships of GDOs with its environment. Therefore gene drives pose numerous challenges to risk assessment and it is currently unclear whether a robust evaluation of potential risks will be feasible in the future, especially when considering the precautionary principle.

In principle, gene drive approaches to genetically modify or eradicate wild populations conceptually challenge nature conservation goals. In addition to a science-based risk assessment, societal and ethical questions of GDO releases must be addressed. The pervasive character of gene drives coupled with the aim to transform or eradicate wild populations and the difficulty to perform field testing indicate the need for a more comprehensive technology assessment. Such an assessment must also address ethical questions regarding nature conservation, e.g. the intention to change the genetic constitution and diversity of wildlife by means of modern biotechnology.

9 ABBREVIATIONS

CBD ..... Convention on Biological Diversity

CISRO ..... Commonwealth Scientific and Industrial Research Organisation

CITES ..... Convention on international trade in endangered species of wild fauna and flora

GDO ..... gene drive organism

GM ..... genetically modified

GMO ..... genetically modified organism

HEG ..... homing endonuclease gene

IUCN ..... International Union for Conservation of Nature

EFSA ..... European Food Safety Authority

ERA ..... environmental risk assessment

EU ..... European Union

MEDEA ..... Maternal Effect Dominant Embryonic Arrest

OECD ..... Organisation for Economic Co-operation and Development

USDA ..... United States Department of Agriculture

WHO ..... World Health Organisation

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