Submission of information on synthetic biology from the Third World Network

1. New technological developments in synthetic biology, including genome editing

New and emerging genome editing techniques are allowing for faster, more targeted, and flexible genetic modification of living organisms. Such techniques can now be applied to almost all species, increasing the scope of potential negative impacts on biological diversity. Their development adds a new dimension to modern biotechnology that is fuelling rapid growth of the synthetic biology field. Their deployment is facilitating and accelerating a myriad of previously unfeasible, complex genetic modifications and manufacturing of biological systems through procedures such as multiplexing and large-scale genome engineering. Despite the growing number of applications being developed and envisaged, the techniques are in their infancy, with critical knowledge gaps and uncertainties remaining on potential unintended effects.

Recent studies have established that widely used genome editing techniques introduce unintended and unexpected effects as an inherent part of the genome editing process. Techniques such as the CRISPR/Cas9 system are increasingly associated with off-target activity, where unwanted modifications are made elsewhere in the genome in addition to the desired change at the target site. Detection techniques such as whole genome sequencing have recently revealed off-target activity in animals (Anderson et al., 2018) and plants (Braatz et al., 2017), challenging previous claims of low or no off-target activity (e.g., Feng et al., 2018; Wei et al., 2018). Further, knowledge gaps remain with regard to the rules of off-target activity, with numerous parameters thought to play a role, including cell type, epigenetics and chromatin environment, sequence of target site and surrounding region and delivery methods, raising uncertainties with regard to predicting and screening for off-target activity. Technical limitations in screening for off-target activity also raise uncertainties with regard to ensuring lack of unintended effects.

Popular genome editing tools have been demonstrated in a recent study to induce unintended genetic alterations at the target site. Any optimisation or improvements in 'specificity' of such systems to minimise off-target activity cannot address these types of unintended effects. Because CRISPR/Cas9 and many other genome editing systems rely on the innate DNA cellular repair mechanisms such as the imprecise non-homologous end joining pathway (NHEJ) to re-join the double-stranded DNA breaks induced by genome editing procedures, this introduces changes to the target site and surrounding region. A 2018 study (Kosicki et al., 2018) demonstrated that CRISPR/Cas9 systems caused extensive genetic damage including small and large DNA insertions and deletions, and complex genetic rearrangements following CRISPR/Cas9 induced DNA breaks. The pattern of changes was variable between edited cells, highlighting the unpredictability of the process. Emerging techniques are being rapidly developed to minimise unwanted effects and to broaden potential applications. However, new genome editing tools such as CRISPR–based 'nickases' designed to circumvent the issue of NHEJ-induced DNA damage, are already being associated with unexpected and unintended genetic changes (Alateeq et al., 2018) despite being promoted as potentially safer and more precise.

Unintended effects at the molecular level may also arise from the process of genome editing that often encompasses the same experimental protocols as first generation genetic engineering, already known to introduce widespread mutations, and epigenetic perturbations, as recently reviewed by Berthaeu (2019).

The abovementioned unintended genetic alterations associated with genome editing could lead to adverse effects including loss of gene function, alteration of gene function, or cause changes in gene expression if unintended changes occur outside of protein-coding regions of the genome, such as in introns, promoters or terminators (see Agapito-Tenfen et al., 2018). Plant allergens are also a major concern and alterations of such allergens may constitute a health risk for human or animal consumption of plant foods.

Recently, CRISPR use in human cells was associated with impacts on cellular regulatory processes such as DNA repair and cell cycle arrest, suggesting unique risk related factors that are unrelated to off-target DNA changes (Ihry et al., 2018; Haapaniemi et al., 2018). This unexpected finding is illustrative of the knowledge gaps regarding the implications of genome editing on cellular function and regulatory pathways, and also of the limited relevance of technical genetic 'precision' as an indicator of safety, or predictability and precision of outcomes.

A growing number of genome editing applications are being suggested for agriculture and conservation despite the above risks and uncertainties, necessitating a broad horizon-scanning process that can capture all these new developments. Suggested applications for conservation include the use of genome editing to introduce barcodes into populations, introducing adaptive traits and somatic modification (Phelps et al., 2019). Widespread genome editing of wildlife has obvious implications for affecting genetic diversity via introduction of desired alterations or unwanted modifications. Such conservation strategies also raise risks of gene flow into non-target organisms. Introducing adaptive traits into populations also has the potential for negative effects on non-target species, for example by rendering them less competitive than the edited organisms. Genome editing is also being promoted as a rapid way to alter orphan and undomesticated crops, previously not so amenable to standard genetic modification techniques (e.g. Lemmon et al., 2018).

The development of novel genome editing methods continues to broaden the number of species that can be genome edited (e.g. Chen et al., 2018). Further, emerging technologies for delivery of genome editing machinery directly into the environment e.g. via aerosol sprays (Zhang et al., 2018), which has thus far been developed for therapeutics but could be applied more broadly, or insects carrying viral vectors, such as developed under the Insect Allies project by the US Defense Advanced Research Projects Agency, also introduces uncertainties with regard to the ability to control exposure.

Developments in other fields such as DNA synthesis are opening up new genome engineering projects that involve synthesising entire genomes such as yeast, assisted by marked advances in DNA synthesis throughput by one-billion-fold in the last few decades. Combined with new and emerging genome editing techniques, applications have been suggested for synthesising entire metabolic pathways, or

even developing completely synthetic genomes that could be applied to agriculture, biofuels, environmental remediation, as well as therapeutics (Chari and Church, 2018), but for which risks have not yet been adequately assessed.

RNA interference (RNAi) technologies are also in development and reaching commercialisation, raising concerns about potential negative effects on biological diversity and human health. It has been established that RNAi molecules acting to modulate gene expression have off-target effects on non-target genes, some of which may be heritable. RNAi products are in development for a wide range of applications including aquatic organism feed, seed treatments and pesticidal sprays (Cagliari et al., 2018). The use of RNAi in the environment is arguably bringing the laboratory process into the field (Heinemann et al., 2018), with attendant risks.

Broad and regular horizon scanning, monitoring and assessment of the most recent technological developments in synthetic biology, including applications of genome editing, are therefore necessary for this fast-developing field, to identify and track new developments, and their potential adverse effects. The outcomes of such a horizon scanning, monitoring and assessment process would need to be reported to the relevant CBD bodies.

2. Current state of knowledge of applications that involve organisms containing engineered gene drives

The development of organisms containing engineered gene drives raises potential threats to biological diversity and also implications for human health and socio-economic circumstances, warranting a precautionary approach.

Recent publications by gene drive developers express concern over the lack of controllability of organisms containing engineered gene drives, with the potential for self-propagating versions likely to be “highly invasive” and spread to most interbreeding populations (Noble et al., 2018). The invasive behaviour of gene drives is an integral part of the strategy for self-propagating versions, and recent work to ensure this characteristic only increases such concerns. For example, the latest gene drive mosquitoes are designed to target and disrupt a highly conserved gene that exists in all *Anopheles* mosquito species across Africa, Asia and South America (Kyrou et al., 2018), chosen in order to limit the evolution of resistance to the gene drive construct. Targeting conserved genes has the potential to affect non-target organisms such as other *Anopheles* mosquitoes via intraspecific breeding or horizontal gene transfer.

The deployment of gene drives for eradicating or modifying entire populations or even species raises huge uncertainties for biological diversity and the wider ecosystems. Altering the course of evolution may have unforeseen consequences for future generations. Yet, limited knowledge exists to be able to predict ecological importance of removing/altering a species. Uncertainties and risks arise regarding, for example, the potential for niche-replacement with disease carrying organisms, changes in behavioural interactions, cascades on food web systems, or unintentionally wiping out organisms that are culturally

or economically important to particular regions of the world or to indigenous peoples and local communities.

Suggested countermeasures such as biological containment and remediation strategies such as daisy-drives, daisy-field, daisy-quorum systems, ERACRs (Element for the Reversal of the Autocatalytic Chain Reaction) and CHACRs (Construct Hitchhiking on the Autocatalytic Chain Reaction) remain largely theoretical and are in their infancy, as acknowledged by developers themselves (Marshall and Akbari, 2018). Furthermore, predictions of efficacy are limited by the lack of detailed understanding of ecological and population dynamics that would be needed to begin to anticipate their effects in wild populations. As noted for mosquitoes, lack of baseline data on dissemination dynamics hampers the ability to predict the spread of mosquito gene drive releases (Eckhoff et al., 2016 supplementary material), which also applies to our ability to predict efficacy of countermeasures. As developers recently warned: "scenarios where homing systems outpace their countermeasures are easily imaginable, and limiting these systems might only be possible in highly contained environments" (Marshall and Akbari, 2018). Currently, huge uncertainties remain regarding our ability to recall gene drives or reverse any unanticipated or unintended effects as a result of a release into the environment.

Geographical containment measures such as island releases for field trials or for general release for applications such as invasive species eradication, are also acknowledged by developers to not be guaranteed methods of containment (James et al., 2018), and as stated by the Ad Hoc Technical Expert Group on Synthetic Biology: 'Islands are not ecologically fully contained environments and should not be regarded as fulfilling the conditions in the definition of contained use as per Article 3 of the Cartagena Protocol unless it is so demonstrated' (AHTEG, 2017: Para 51 (b)). Nonetheless suggestions to field trial gene drive mosquitoes on islands in Uganda are being investigated (Lukindu et al., 2018).

Gene drive systems such as CRISPR homing endonucleases also have the potential to generate unintended, heritable off-target effects (as raised above with regards to genome editing) that may go on to generate novel genotypes and phenotypes, for example enhancing capacity to transmit disease (in the case of disease vector gene drive systems), toxicity to predators, and wider impacts on food webs and ecosystems (Hayes et al., 2018).

Using gene drives for controlling disease vectors also raises serious health concerns regarding the potential interruption or loss of acquired immunity to disease. Uncertainties remain regarding the potential for gene drives to control vector numbers in the long term, if issues such as resistance arise. The complex epidemiology of diseases such as malaria may be adversely affected if malaria vector numbers return once immunity has been lost.

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