Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety – Submission of information by Third World Network

a) Experience in undertaking risk assessment of living modified organisms containing engineered gene drives; or else, lack of experience in doing so

There is a lack of experience in undertaking risk assessment of living modified organisms (LMOs) containing engineered gene drives, primarily because such LMOs are currently at the research phase in laboratories and as yet, there is no known release into the environment. However, even at the contained use stage, risk assessments are necessary and there are experiences with pathogens and LMOs in contained use and that can inform such a risk assessment for LMOs containing engineered gene drives. This becomes particularly important as there is a risk of escape of LMOs containing engineered gene drives from contained use facilities, necessitating stringent oversight of contained use activities. Nonetheless, risk assessment of LMOs containing engineered gene drives may have to adapt the standards set out for pathogens and LMOs in contained use (Simon et al, 2018), due to the challenges posed by the spread and persistence of LMOs containing gene drives. (See paragraph 1 of point (c) below for a further discussion of specific needs in relation to contained use).

LMOs containing engineered gene drives are distinct from current LMOs and there are increased complexities associated with their characteristics and ecological impacts. It would be inappropriate to directly extrapolate experience of risk assessment of current LMOs to that of LMOs containing gene drives, without considering their particular features and associated risk assessment challenges. As identified by Simon et al. (2018), LMOs containing gene drives can be distinguished from current LMOs due to numerous novel features, including: 1) outcrossing and spread of the transgenes as a prerequisite for gene drives; 2) transferring the laboratory to the field, with inheritance of the genetic modification toolbox occurring in an LMO containing engineered gene drives; 3) the modification of wild populations as opposed to cultivated plant species; 4) the transition from indirect (modification against stressors) to direct modification of stressors such as pest species; and 5) modification of common goods. These features have implications and pose challenges for risk assessment, some of which are expanded upon below.

b) Challenges experienced or foreseen in undertaking risk assessment of living modified organisms containing engineered gene drives

1. The issue of spread raises fundamental challenges for risk assessment, with the potential, in the case of 'global' gene drives, for just a few LMOs containing engineered gene drives to establish themselves in the environment. Concerns over this lack of controllability has been raised by gene drive developers and reiterated by biosafety experts, who have warned that such gene drives are likely to be "highly invasive" and could spread to most interbreeding populations (Esvelt and Gemmell, 2017; Noble et al., 2017; Simon et al., 2018).

There remains disagreement, including at the Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology, as to the utility of conducting the risk assessment in a stepwise manner, that is, from contained use, to field trials and finally to open releases, with the results at each step informing the next step of the risk assessment, an approach that is common for LMOs. It is our view that such an approach is not appropriate at this stage of uncertainty about the impacts of LMOs containing engineered gene drives on the environment, as it includes field-testing, which requires release into the environment, and could transform into a full-scale release. The AHTEG on Synthetic Biology

likewise highlighted that “the step of release into the environment might be irreversible”, and therefore called for a precautionary approach.

Suggestions to perform field trials on islands are insufficient as a containment measure, as even so-called isolated releases of LMOs containing gene drives, may lead to further spread (e.g. wind-blown mosquitoes or rats on cars, boats, planes etc.). 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”. This invasive nature of gene drives also raises serious concerns for transboundary movement and highlights the need to obtain the free, prior and informed consent of potentially affected indigenous peoples and local communities.

For applications such as the eradication of invasive species from their non-native habitat e.g. island rodent populations, the issue of potential escape also raises risk assessment challenges. The spread dynamics of the gene drives is dependent on numerous factors including migration and reproduction parameters of the target population, over time and space, which will be challenging to predict prior to environmental release.

Lastly, assessing outcrossing of transgenes to closely related taxa as is common for risk assessment for current LMOs, will have to take into consideration the fact that outcrossing potential is much increased for LMOs containing gene drives. Unlike current LMOs, those containing gene drives do not need a selective advantage in order to spread. While transgene spread from current LMOs has a high chance of being eventually lost, this is not the case for LMOs containing gene drives. Assessing the outcrossing potential of LMOs containing gene drives is further complicated by a lack of baseline data on genome sequences of potential outcrossing partners to be able to estimate the likelihood of outcrossing.

2. The issue of transferring the laboratory to the field also raises risk assessment challenges for predicting unintended effects prior to environmental release. In contrast to current LMOs where the finished product is constructed in the laboratory, gene drives perform the genetic modification in the wild, altering the germline at each generation. This raises additional uncertainties regarding how they will behave in a genetically diverse, wild population with the added complexities of potential unintended molecular effects e.g. heritable off-target effects (Hayes et al., 2018); ride along of additional sequences (Courtier-Orgogozo et al., 2017); toxicity of the genome editing machinery; and resistance development. How such complex genetic modification processes occurring continuously over time and space will affect issues such as outcrossing potential, molecular aspects e.g. genome stability, transgene stability/efficacy, or wider ecological impacts e.g. ecosystem function and species interactions, will not have been considered to the same extent as with current LMOs.

3. Gene drives are being developed for the modification of wildlife, transitioning away from current LMOs that are focused on cultivated plant species that are bred to behave uniformly. This has major consequences for potential effects that will go beyond agroecosystems. Even for agricultural gene drive applications such as pest species eradication, the definition of a pest is relative to specific economic interests and ecological systems (Courtier-Orgogozo et al., 2017). The movement of such LMOs containing gene drives into ecosystems where it is no longer considered a pest, could potentially occur. Modification of wildlife thus opens up challenges with regards to assessing novel ecosystems or receiving environments and ranges of potentially affected organisms. For example, outcrossing into wildlife may occur in ways that are rarely characterised for current LMOs.

Further, current genetic modification strategies have thus far been designed to work against stressors, such as designing crops to confer pest resistance. In contrast, LMOs containing gene drives

are being developed to work directly against a stressor i.e. to suppress a pest or disease vector, again altering the ecological burden to species and habitats that go beyond the agroecosystem (Simon et al., 2018).

4. With regard to performing risk assessment for intended effects of global gene drives designed to modify or eradicate entire populations or species, effects on wider ecosystems are very difficult to predict and potentially harder to reverse, with experts nonetheless warning about severe ecosystem effects (Hochkirch et al., 2017). Further, long-term ecological effects may take decades to appear, making proper problem formulation, data acquisition, modelling and/or practical testing challenging. Altering the course of evolution may have unforeseen circumstances for future generations. Currently, limited knowledge exists to be able to predict the ecological importance of removing/altering a species, particularly wild populations such as weeds and mosquitoes. Modelling studies thus far have largely focused on efficacy instead of assessing risk, and are currently limited by lack of baseline data. For example, with regards to mosquitoes, gene drive releases will likely involve the release of males, but mosquito studies have overwhelmingly focussed on females, so that data on male population size, survival and movement are currently limited.

There is potential for knock-on effects on the wider ecosystem affecting food webs such as pollinator, predator or pest numbers; niche replacement where a new species takes over the environment left behind by an eradicated species, including for example, another disease-carrying mosquito species; or unintentionally wiping out species that are culturally or economically important to particular regions of the world or to indigenous peoples and local communities.

5. At the molecular level, there are several risks associated with the gene drive technologies that could also introduce unintended effects on ecosystems and human health. Gene drives developed with genome editing tools such as CRISPR/Cas systems can introduce heritable off-target changes to the genome of the gene drive organism (Hayes et al., 2018). Studies have recently highlighted such unintended effects including unintended mutations, complex rearrangements, translocations, insertions and deletions (Kosicki et al., 2018) and, horizontal gene transfer of foreign DNA sequences into CRISPR-induced double-stranded breaks (Ono et al., 2019). Unwanted changes to DNA may go on to alter phenotypic characteristics of organisms such as enhancing capacity to transmit disease in the case of disease vector gene drive organisms such as mosquitoes, or altering toxicity to predators, for example. Such off-target effects are difficult to predict and characterise before the release into the environment, particularly in genetically diverse wild populations.

6. Uncertainties also surround the potential scenario of multiple LMOs containing gene drives being released into an environment, raising concerns that potential interactions could have unforeseen adverse ecological effects. As raised by Courtier-Orgogozo et al., (2017), such a scenario "is likely to cause unpredictable ecological disturbances with far reaching consequences". These uncertainties make risk assessment even more challenging.

c) Specific needs (if any) to properly undertake risk assessment of living modified organisms containing engineered gene drives

1. Strict conditions on contained use for LMOs containing engineered gene drives are warranted considering their potential to spread and persist from the unintentional release or escape of a single organism. Strict conditions need to implement multiple strategies as it is possible that "any single confinement strategy could fail" (Akbari et al., 2015), including molecular, ecological, reproductive or physical measures.

Such an approach has been acknowledged by the AHTEG on Synthetic Biology, which has pointed out that the development and implementation of well-designed strategies, which include physical containment, might be needed for the organisms, components and products of synthetic biology (including LMOs containing engineered gene drives) under contained use, in order to effectively limit their survival or spread and to prevent or minimize their exposure of the environment.

The basic idea for regulating contained use activities is to set ascending levels of containment, which correspond to increasing levels of protection. Establishing the appropriate biosafety level requires a risk assessment. Applied to LMOs containing engineered gene drives, those with a high potential for spread or invasiveness should be subject to higher containment stringency and management procedures (Benedict et al., 2018; van der Vlugt et al., 2018). Current contained use measures, as applied to pathogens, may include some that are not relevant for LMOs containing engineered gene drives, and others that may not provide adequately for the suite of controls necessary to contain such LMOs. This means that there is a need to adapt the details accordingly, along with an additional focus on potential environmental hazards due to potential species and ecosystem effects (Simon et al., 2018).

2. Due to inability to perform a stepwise risk assessment as conducted with current LMOs, there is an added need for alternative assessments under contained use. Modelling studies offer a safe method for predicting effects – and even prior to modelling, the acquisition of baseline data that feed into modelling parameters is needed. Such modelling studies need to move away from focusing on efficacy as has occurred to date, to also focus on ecological and health risks that incorporate disease epidemiologies for public health applications, in the case of gene drive mosquitoes, for example. Strict contained use studies such as long-term caged-studies, including those done in simulated environments can also inform on potential risks, unintended effects and efficacy issues such as resistance development. Further, incorporation of data from other fields, such as past experiences with current LMOs and population eradication programs for invasive species, could also be incorporated.

3. Monitoring and detection of LMOs containing engineered gene drives will become particularly important because of the challenges foreseen with risk assessment, which may mean that there could be more gaps in knowledge and uncertainties. Monitoring and detection will need to take on board multiple considerations. For example, measuring of potential outcrossing needs to be thorough. It is possible that detection of a gene drive construct may be confused with fragments of inactive gene drives rendered non-functional by unintended effects such as resistance development, but are nonetheless inherited. Careful protocols such as the use of whole genome sequencing for accurate detection methods are thus warranted. Monitoring of non-target organisms for detection of outcrossing events would also be needed. Detection of both intended and unintended ecological effects on the environment, would also be required, even in the event of localised gene drive systems, or with gene drives that have since vanished.

5. Cost-benefits analyses of gene drive technologies are also critical for evaluating whether or not gene drive technologies are suitable and can complement the risk assessment, assisting in decision-making. Recent modelling papers suggest that limitations in efficacy may necessitate multiple and regular releases of LMOs containing gene drives (Eckhoff et al., 2018). Such analyses are necessary to be able to adequately assess their potential utility as a public health strategy against malaria for example where the LMO containing gene drive will create a common good in the form of disease control. As raised by Simon et al., (2018), public goods need to be evaluated against public burden that may arise from their ecological and socio-economic impacts both for current and future generations.

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