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# Submission of information and supporting documentation relevant to risk assessment of gene drives

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## Introduction and summary

This backgrounder was compiled for submission of information to the Open-ended Online Forums on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety (SCBD/CPU/DC/WM/MAQ/MW/90762) within the proceedings of the Convention on Biological Diversity (CBD). It provides an overview from the perspective of the protection goals, such as health and the environment.

The most relevant finding is a strong increase in SynBio applications focusing on Living Modified Organisms (LMOs) containing self-propagating artificial genetic elements, such as gene drives that are intended to actively spread technically inserted genetic elements within domesticated or non-domesticated populations. These applications are meant to move the process of genetic engineering from the laboratory into the fields.

Recent experiments with the X-shredder application in *Anopheles* highlight the need for in-depth risk assessment on a case-by-case basis, including questioning the assumptions made by experts involved in the development of the LMOs. In the above experiment, field trials were announced with genetically engineered mosquitoes based on incorrect assumptions regarding the strain and the insertion site of the artificial gene constructs. If released, this application could have devastating consequences for both the target and non-target species.

It further shows that ‘cut-off’ criteria are essential in the risk assessment of organisms developed to actively spread artificial genetic elements. Such criteria could facilitate decision-making when faced with numerous unknowns. The regulators, therefore, need to prioritise both a prospective technology assessment and investigations into systemic risks. The concepts of nature conservation and environmental protection are largely based on the principle of avoiding interventions that, for example, could damage natural self-organisation capacities in protected areas or that could compromise sustainability criteria relevant for land use. These concepts must also be upheld in the field of genetic engineering and gene drives.

## 1. SynBio applications with self-propagating artificial genetic elements as gene drives

There has been a strong increase in SynBio applications for LMOs with self-propagating artificial genetic elements (SPAGE) as gene drives (see von Gleich & Schröder, 2020). These applications are intended to actively spread technically inserted genetic elements within domesticated or non-

domesticated populations. They involve a move from the laboratory to the fields and go beyond the applications of gene drives (Adelman, 2021; BfN, 2022).

## 1.1 Gene Drives

A report issued by the Federal Agency for Nature Conservation (BfN, 2022) gives a short overview of some technical characteristics: SynBio gene drives involve incorporating genetic engineering tools (e. g. CRISPR/Cas) as a part of the genetic modification. If organisms with synthetic gene drives are released, these genetic engineering tools are also released – one might say that the genetic engineering experiment is moved into the environment to become a “lab-in-the-field” (Simon et al., 2018). Such gene drive organisms can interbreed with their wild relatives, and thus affect inheritance. More than half, and possibly all, of the offspring will inherit the genetic modification (including the genetic engineering tools). Without the gene drive, Mendelian inheritance laws mean that only half of the offspring would inherit the modification.

Gene drives are intended to enable genetic modifications to persist more successfully in wild populations over time, and to become prevalent under certain circumstances. However, the technique used in developing synthetic gene drives theoretically permits LMOs to spread even if they possess characteristics which are disadvantageous to the organism and/or its reproductive system (for instance, only producing male offspring). It can even result in the collapse or extinction of a population.

Currently, there are two basic SynBio gene drive concepts: “suppression drives” that are meant to introduce genetic elements to reduce or eradicate natural populations, for example, by interfering with their capacity to reproduce (Kyrou et al., 2018; Hammond et al., 2021); “replacement drives” that are meant to replace natural populations with persistent GE populations with altered biological characteristics and inheritable artificial genetic elements (Gantz et al., 2015; Carballar-Lejarazú et al., 2020; Green et al., 2022; Adolfi et al., 2020; Devos et al., 2021).

The target organisms include mosquitoes (e. g. Gantz et al., 2015; Hammond et al., 2021; Kyrou et al., 2018), flies (Ni et al., 2021; Yan et al., 2021; Kaduskar et al., 2022), rodents (Grunwald et al., 2019; Bunting et al., 2022), mites (Faber et al., 2021), plants (Siddiqui et al., 2021; Zhang et al., 2021; Barret et al., 2019; Tek & Budak 2021) and yeast (DiCarlo et al., 2015). Future applications may involve snails (Grewelle et al., 2020), wasps (Meiborg et al., 2023) and diamondback moths (Xu et al., 2022). Other applications under consideration are coral reefs, grey squirrels, the crown-of-thorns starfish, feral cats, cane toads, signal crayfish and brushtail possums (for overview, see: Hartley et al., 2022). Some of the organisms being considered for these applications could facilitate gene flow between different, related species (Taylor et al., 2001; Weetman et al., 2014; Wolf et al., 2023).

## 1.2 SynBio Viruses

There are further SynBio applications that are designed to actively spread technically inserted genetic elements within domesticated or non-domesticated populations. Again, these applications involve a move from the laboratory to the fields.

The abovementioned BfN (2022) report includes an overview of some of these applications: genetically engineered viruses are currently being developed for a number of different purposes, with increasing risks to health and the environment (Lentzos et al., 2022). In order to introduce the viruses into their target organisms, current research is looking at ways of spreading the viruses via

insects, and thus transfer them to plants. The aim of these applications is to allow genetic modifications to be rapidly introduced into the plants of an existing population, without having to rely on reproduction ("horizontally"). This method, which is also referred to as Horizontal Environmental Genetic Alteration Agents (HEGAAs) (Reeves et al., 2018; Frieß et al., 2020; Pfeifer et al., 2022), is being developed as a crop protection strategy and funded by the Defense Advanced Research Projects Agency (DARPA) at the US Ministry of Defense. Specific viruses are being developed for use in plants (Gentzel et al., 2022; Nagalakshmi et al., 2022). This and other virus-based strategies are also being discussed in connection with environmental and nature conservation (Lentzos et al., 2022). Other applications are being developed to use viruses to change the genome of gut bacteria (Lam et al., 2022).

### 1.3 SynBio LMOs with the potential to spread genetic information

Other applications, which (at least to some extent) are expected to spread genetic information within undomesticated populations, include constructs to suppress or disrupt populations of flies (Ant et al., 2012) or mosquitoes (Windbichler et al., 2008; Evans et al., 2019; Waltz, 2021) by introducing lethal gene constructs. Some studies also demonstrate an interest in establishing genetic engineering mechanisms that are inheritable to following generations (Impens et al., 2022).

Applications are meanwhile being developed to engineer bacteria and fungi that are part of the plant rhizosphere (Shelake et al., 2019; Shulse et al., 2019; Temme et al., 2012; Ke et al., 2020; Shekhawat et al., 2022; Shanmugam et al., 2019), or animal microbiome (Bilgo et al., 2017; De Vooght et al., 2014; Fang et al., 2011; Gilbert et al., 2016; Leonard et al., 2018; Lovett et al., 2019; Leonard et al., 2020; Rangberg et al., 2012; Ren et al., 2008; Lam et al., 2021), or symbiotic in corals (Levin et al., 2017). After release, such applications would allow SynBio LMOs to persist, spread and propagate over longer, maybe even unlimited, periods of time.

In this context, further SynBio or transgenic applications in trees (Ahuja, 2009; Bauer-Panskus et al., 2020; GeneWatchUK, 2020; NAS, 2019; Wang, 2004; Zhang et al., 2013; Zeeman & Solhaug 2022; Wang et al., 2022; Tao et al., 2022) or fish (Moreau et al., 2011; Sundström et al., 2014; Devos et al., 2019; Vandersteen et al., 2019; Magalhães et al., 2022) should also be taken into account, as they have the potential to facilitate unintended geneflow into wild populations.

## 2. Risk assessment and risk management considerations

The following section takes a closer look at the example of the X-shredder application in *Anopheles*, and shows that in-depth risk assessment will be needed in each and every instance, including questioning assumptions made by experts involved in the development of the LMOs. It further addresses the need to consider cumulative effects and potential interactions. In addition, it makes the case for the risk manager to prioritise a prospective technology assessment and evaluate systemic risks.

### 2.1 Lessons learned from the 'X-shredder' experiment

A sex distorter system called the 'X-shredder' was used in *Anopheles* resulting in a 95% male bias among the progeny of transgenic males (Galizi et al., 2014). The X-shredder is a homing endonuclease called I-PpoI, which is expressed during male spermatogenesis and cleaves (i. e. destroys) the X-chromosome at multiple sites resulting in only Y-chromosome bearing sperm cells, and thus predominantly male offspring. The X-shredder in this form is not considered to be a gene

drive, as the transgenic construct of the X-shredder is supposed to be inherited according to Mendelian rules. It can be regarded as ‘self-limiting’ as population suppression is only possible by continuously releasing mosquitoes bearing the X-shredder construct. The progeny of a transgenic male containing the X-shredder construct are thus predominately male, with half of the male progeny themselves being transgenic since they carry the X-shredder construct.

The Target Malaria consortium also conducted caged trials using the mosquitoes with a self-limiting version of the X-shredder. The mosquitoes were reared in small cages, as described by Galizi et al in 2014. Since the results from larger caged trials seemed promising, (Facchinelli et al., 2019; Pollegioni et al., 2020), the release of the mosquitoes was announced for the upcoming years.

The releases of the mosquitoes inheriting this self-limiting X-shredder are planned as ‘phase two’ releases. The trials are intended to be a follow-up to ‘phase one’ releases conducted in 2019 that were performed with transgenic mosquitoes (Target Malaria, 2019). In the phase one releases, a first version of the X-shredder (Windbichler et al. 2008) was used that caused male sterility in the released mosquitoes, and was thus was supposed to prevent any offspring. There are now plans for ‘phase two’ releases with the ‘self-limiting’ version of the X-shredder. These trials are supposed to be followed by final ‘phase three’ releases of self-sustaining gene drives (Kyrou et al., 2028; Simoni et al., 2020) in future.

However, in 2022, it became known that the trials were planned on incorrect assumptions regarding the strain and insertion site of the artificial gene constructs: Vitale et al. (2022) showed that the preliminary assignment of the transgene location (Galizi et al., 2014) on Chromosome 3 R 36D was incorrect. It seems the presence of highly repetitive sequences at the insertion site led to the wrong conclusions. Only after DNA sequencing analysis and in-situ hybridization was it possible to clearly identify the integration of the transgene in a centromeric region of Chromosome 2 R 19D. The researchers (Vitale et al., 2022), therefore, made a point of emphasising the need for accuracy in genome sequencing data for organisms of medical importance, such as *Anopheles* mosquitoes, and other available tools that can support genomic locations of transgenes. They also noted that the genome assembly of species with highly polymorphic genomes, such as mosquitoes, can be challenging.

The actual site of insertion may need further consideration: insertion in a centromeric region may have implications for the overall stability of the genome and the function of the X-shredder, as recombinations and mutations in centromere regions are generally known to be potentially associated with chromosome segregation errors and aneuploidy (Nambiar et al., 2016).

Further incorrect assumptions were made in the Target Malaria Consortium trials: although the G3 strain that was used in the trials has for decades been classified as *Anopheles gambiae*, it is now thought to be a hybrid between *Anopheles gambiae* and *Anopheles coluzzii* (Pollegioni et al., 2023). This has major implications: as Pollegioni et al. (2023) states, *Anopheles gambiae* and *Anopheles coluzzii* are two major African malaria vectors, morphologically indistinguishable but characterised by a widespread genomic divergence. These ‘sister species’ differ in their ecological niche partitioning at the larval stage and in swarming behaviour, favouring their assortative mating. Therefore, risk assessment as performed before the release of the X-shredder LMOs assumed that gene flow from the genetically engineered strain (supposed to be *Anopholes gambiae*) to other species (such as *Anopheles coluzzii*) would be unlikely. This assumption shows a lack of awareness of the real genetic background of the strain and its potential impact on its ecological behaviour. If a hybrid strain was used in the field trials, no conclusions can be drawn on the likelihood of further geneflow just from the characteristics of the parental species.

Furthermore, Pollegioni et al. (2023) conducted experiments with the X-shredder in two recipient colonies of *Anopheles coluzzii*. The crossing experiments revealed that the transfer of genetic material from one species to another (rate of introgression) behaved differently than normally expected. The introgression is probably influenced by the genomic location of the transgene. This can have multiple implications for disease transmission as well as ecological adaptation. This underlines the importance of assessing potential gene flow, crossing and next generation effects (Then et al., 2020) in risk assessment prior to any releases. Pollegioni et al. (2023) also suggest a thorough assessment with an extended backcrossing strategy for any X-shredder candidate strain for field release – to be used as a regulatory step for the evaluation of potential risks of the genetically engineered mosquitoes, fitness and insecticide resistance coupled with population suppression effects.

In this context, it is important to be aware of the fact that *Anopheles gambiae* and *Anopheles coluzzii* belong to a group of at least nine 'sibling' species of mosquitoes which can interbreed and are capable of gene flow. Six of them are known to vector human malaria. Hybridization between these species can yield fertile hybrids (Connolly et al., 2021). Therefore, if experiments are conducted with gene drives or other mechanisms that actively spread artificial gene constructs, then next generation effects (Then et al., 2020) may occur from a wide range of crossings and backcrossing that need to be assessed prior to any release.

Connolly et al. (2021) explored pathways to potential harm from the release of a population suppression gene drive to control the human malaria vector, *Anopheles gambiae,* in West Africa. They attempted to address some of these problems in their proposal on the risk assessment of gene drive mosquito, stating that the environmental risk assessment of the release of a genetically engineered organism "*needs to consider both direct effects on individual organisms that the transgenic itself generates, such as via predation, competition, habitat alteration, hybridization and introduction of new parasites and diseases, and indirect effects such as those on individual organisms in the wider environment without immediate contact with the transgenic.*"

At the same time, Connolly et al. (2021) seem to have overlooked the fact that not all nine sibling species of *Anopheles gambiae* can be considered to be target species, as not all of them are a vector for malaria transmission. Furthermore, they appear to simply assume that unexpected next generation effects (Then et al., 2020) do not need to be considered if there is an efficient suppression of the target population. However, given the wide range of genetic diversity within the nine species, it is hardly possible to calculate efficiency in suppressing a population. In addition, as the example of the X-shredder shows, any prediction regarding the gene function of the inserted genes may be compromised by incorrect assumptions, such as the gene insertion site. Consequently, what may be started as a suppression gene drive or a self limiting gene construct may end as a persisting and spreading artificial genetic element within wild populations, causing long-term unexpected and potentially harmful effects.

The 'Anopheles gambiae 1000 Genomes Consortium' (2017) findings also have to be taken into account in this context. They "*revealed complex population structure and patterns of gene flow, with evidence of ancient expansions, recent bottlenecks, and local variation in effective population size.*" They warn that "*the design of new tools for mosquito control using gene-drive systems will need to take account of high levels of genetic diversity in natural mosquito populations.*"

In summary, as the X-shredder example shows, incorrect assumptions and a lack of awareness of unexpected effects may have devastating consequences for both target and non-target species, especially after large scale releases.

Empirical studies are needed to examine the genetic function, the genetic stability and the ecological impact of further crossing and hybridization, as they cannot be predicted from the intended properties of the LMOs created in the lab and reared in cages. Risk assessment faces enormous challenges if next generation effects, that may occur from crossing with the nine interrelated 'sibling' species, are to be taken into account. Therefore, any risk assessment of organisms intended to actively spread technically inserted genetic elements within domesticated or non-domesticated populations, will need 'cut-off' criteria to allow decision-making in the face of numerous unknowns (Then et al., 2020).

## 2.2. How to address cumulative risks?

As already mentioned, there has been a strong increase in the number of SynBio applications for LMOs that are intended to actively spread technically inserted genetic elements within domesticated or non-domesticated populations. These applications go beyond the applications of gene drives. The following paragraphs, therefore, provide a more in-depth explanation of self-propagating artificial genetic elements, i. e. SPAGE (see von Gleich & Schröder, 2020).

Indirect, delayed and cumulative adverse effects arising from releases of SPAGE may be more or less likely, depending on their specific biological characteristics (intended or unintended) and the regional distances. Large scale releases may increase the likelihood of such effects and transboundary movements. Given the wide range of the applications listed above, legal requirements for assessing cumulative and long-term effects are urgently needed.

There are at least two categories that should be taken into account:
(1) Cumulative effects of SPAGE involving several species: environmental risk assessment that only takes individual LMOs into account may fail to predict or assess long-term cumulative effects, or possible interactions with the receiving environment and/or other SynBio-LMOs. Consequently, although releasing low numbers of individual LMOs for a short time may possibly not result in adverse effects in an ecosystem, the combination with other SynBio LMOs or the release of larger numbers of a specific SynBio LMO over a longer period of time might lead to a 'tipping point' being passed and cause irreversible damage. These cumulative effects may, for example, also be caused by interactions between SynBio LMOs, which would pose huge challenges of potentially extreme complexity in risk assessment.

(2) Cumulative effects resulting from releases of different SPAGE applications within the same species or within 'sibling' species: suppression drives and replacement drives are, for example, currently being developed in *Anopheles*. This will impact several species that may cross with each other. Any crossing may result in new combinations of the artificial genetic elements that were neither expected nor subjected to risk assessment.

In general, it is difficult to predict any effects resulting from interactions between SynBio organisms (such as gene drive organisms), as they may be additive, antagonistic or synergistic. Such effects may be dependent on specific combinations of the traits and/or exposure to stressful conditions. Even if each of the traits were to be considered 'safe', uncertainties or even unknowns can still emerge from the combination of the traits. Therefore, environmental risk assessment of the single

traits may fail to predict or assess either short- or long-term cumulative effects, or possible interactions with the receiving environment, or several traits in combination. However, the general problem with cumulative and combinatorial effects is that, so far, no established methodology is in place that would allow robust risk assessment before the release. It should also be taken into account that these effects include or promote transboundary movements.

In addition to risk assessment, a comprehensive and prospective assessment is essential in order to address systemic risks to biodiversity. Similar to environmental pollution with plastics and chemicals, it is not always an individual SynBio LMO which may create the real problems, but rather the sum of diverse effects on the environment. Environmental problems created by the release of SynBio LMOs (such as gene drive organisms) may last as long as or longer than those caused by plastics and pesticides – thus impacting future generations and transboundary movements.

Therefore, it is vital (for the risk manager) to generally restrict the number and scale of SynBio LMOs releases into the environment in order to prevent unintended transboundary movements. Similar control mechanisms are needed to deal with potential cumulative adverse effects on health and the environment, and to avoid passing potential tipping points that would cause irreversible damage in ecosystems. The concepts of nature conservation and environmental protection are largely based on the principle of avoiding interventions that, for example, could damage natural self-organisation capacities in protected areas or that could compromise sustainability criteria relevant for land use. These should also be applied in the field of genetic engineering, for SynBio LMOs and, in particular, gene drives and other SPAGE.

## 3. Further supporting information and conclusions

Kuzma (2022) states that gene drive organisms (GDOs) "*have features of 'emerging risks' that are 'characterized mainly by uncertainty regarding their potential consequences and/or probabilities of occurrence' which 'can be due to a lack of knowledge about causal or functional relationships between new risk sources and their environment or to the insufficient application of available knowledge to the case in question' (IRGC, 2015). For these situations, evaluating the 'substantive validity' of risk assessments - where outcomes of the risk assessment are compared to what happens in reality - is not feasible, especially prior to any environmental release. Therefore, 'procedural validity' of the risk assessment, that is how the risk assessment is conducted, becomes even more important than attempting to ascertain the substantive validity of particular risk evaluations prior to GDO release and field data collection.*"

Warmbrod et al. (2022) also address the problems in predicting unexpected hazardous effects: "*A major risk of gene drives is the potential unknown consequences of unpredicted spread or interactions; avoiding interactions between different gene drive organisms should be a paramount priority as potential interactions expand the risk of unknown consequences.*"

Frieß et al. (2023) warn that modelling approaches also might fail to be sufficient to predict and assess all relevant risks: "*In our review, we analyse the scope and structure of existing models to examine how they may assist the ERA. Our analysis reveals that a majority of models so far are deterministic, non-spatial and not tailored for a specific target organism. Models often use simplified assumptions on the biology of the species and seem to be made to test the effectiveness of the drive. Few models go beyond this and verify whether model predictions may be realistic under field conditions. We identified four advanced models that we judged to be the most ecologically*

*realistic and compared the implemented parameters with ERA requirements by the European Food Safety Authority (EFSA) and World Health Organization (WHO) for genetically modified insects and mosquitoes. Although a number of abiotic and biotic factors are already considered in these models, mating-related factors and traits relevant to the interactions between the GMO and target organisms and with other species are largely excluded. Overall, our results show that biological and ecological realism are still poorly realized in current models and that most models aim to predict efficacy rather than ecological effects. Given the complexity of natural ecosystems, it may not be possible to compile a single model to cover all complexities. Thus, models should be further developed with the purpose to assist specific questions related to the risk assessment of GDs. Moreover, uncertainty will be a key issue for any model used in RA and we see the need to improve this aspect when modelling gene drives."*

There are several publications that address the complexity and specificities of risk assessment for genetically engineered gene drives (e. g. EFSA, 2013; EFSA, 2020; CSS, 2019; von Gleich & Schröder, 2020, Frieß et al., 2019; Simon et al., 2018; Dolezel et al., 2019; Devos et al., 2021; Champer et al., 2021; Connolly et al., 2021; Kuzma, 2022; Conolly et al., 2023; Verma et al., 2023; Frieß et al., 2023).

For example, EFSA (2013) identifies several categories of long-term effects which reflect increases in spatial and temporal complexity. EFSA notes that only certain spatial and temporal scales can be empirically tested before releases take place, hence there may be long-term effects as a result of increased spatial or temporal complexity after being brought to market. Examples include interactions of LMOs with other species (including pathogens), as the complexity of species interactions increases with spatial complexity. EFSA also mentions that, over longer periods of time, evolutionary, behavioural and other changes in species will cause further changes in species interactions. Climate also differs across spatial and temporal scales: increasing spatial complexity increases the combinations of environmental variables that gene drive organisms are confronted with. Increasing temporal complexity also increases the range of environmental variables that gene drives are confronted with, e. g. as a result of climate change. Climate change affects other LMOs and natural species, thus also affecting species interactions. In fact, climate change is likely to alter whole communities of different species.

In the case of gene drive organisms (and more generally, SPAGE application), there are generic difficulties in collecting the relevant data for problem formulation, including identification of hazard and exposure pathways. Sufficiently robust and comprehensive data, such as population dynamics, life cycle, life history traits, the environments, ecosystems, next generation effects and potential evolutionary impacts, might not always be available. Such problems may in more detail concern:

- Assessment of the spatio-temporal dimension of releases of gene drive-inheriting organisms and resulting environmental exposure;
- Effects in offspring due to the self-factoring of gene drives in wild populations over dozens of generations in wild populations with a wide range of genetic backgrounds;
- The complexity of environmental x genome interactions in a broad range of heterogeneous environments;
- The complexity of environmental x genome interactions under significant changes in the environment, such as the climate change;
- The problem of deriving sufficient data on the role of the target organisms in ecology;
- The problem of deriving sufficient data on the impact on non-target organisms and human health;
- The problem of identifying adequate comparators;

- The problem of deriving robust data to predict long-term effects;
- The problems in performing long-term case-specific monitoring;
- The lack of availability of sufficiently effective methods to prevent and / or mitigate adverse effects if observed after release.

The increase in spatial and temporal complexity associated with the release of genetically engineered gene drive organisms is likely to decrease the robustness of the environmental risk assessment (ERA), especially if several generations are involved (Then et al., 2020). If the persistence of these organisms cannot be delimited in terms of time and space, ERA has to consider long-term dimensions, e. g. by addressing the alteration of its gene drive mechanism under evolutionary pressure. Evolutionary processes make it possible to turn events with a low probability of ever happening into events that are likely to happen (Breckling, 2013). Inherent non-knowledge can, thereby, increase to such an extent that the conclusiveness of risk assessment is severely affected. In many cases, there will also be no possibility of establishing a suitable control or re-call strategy. The key question is: How can non-knowledge (see Böschen, 2009), uncertainties, or incertitude caused by limitations of scientific knowledge and knowledge production systems be integrated into a regulatory system of decision-making?

It has to be assumed that at a certain point in the dissolution of spatial and temporal boundaries, it will become necessary to apply cut-off criteria within the risk assessment process, to decide if its outcome will be sufficiently reliable and conclusive. The introduction of the cut-off criterion for spatio-temporal controllability as an additional step in ERA can be used to delineate some of the boundaries between knowns and unknowns considered to be crucial (Then et al., 2020). This additional step will foster the robustness of risk assessment and can substantially benefit the reliability of decision-making within approval processes. It is necessary for preventing any release of SPAGE which are not sufficiently risk assessed: if it is likely that the organisms can escape spatio-temporal controllability, the risk assessment cannot be sufficiently reliable because it is not conclusive. Under such circumstances, the environmental release of the gene drive organisms (or SPAGE) would not be compatible with the precautionary principle.

The precautionary principle allows for new risks to be taken, but only as long as effective measures are available and can be implemented if something ‘goes wrong’. Such measures depend on being able to control the release of LMOs in their spatio-temporal dimension. Therefore, any release which cannot be sufficiently controlled in space and time is in contradiction to the precautionary principle and cannot be allowed to happen. This finding is especially relevant for genetically engineered gene drive organisms and other SPAGE.

It should be emphasised that, in terms of the precautionary principle, post-marketing monitoring cannot replace adequate risk assessment or the need for sufficiently effective methods to prevent and / or mitigate adverse effects if observed after release. Gene drives will have an evolving post-release phase over space and time, which might be impossible to monitor adequately. Capacity and resources for long-term monitoring might in many cases create substantial problems. Further, the transboundary issues of monitoring and response also need to be addressed, planned and resourced. Lack of reversibility will also be an issue in many cases.

Monitoring is further dependent on the data collected prior to the release. However, assessing potential risks requires that the difficulties related to data collection and analysis are taken into account, such as obtaining sufficiently robust and comprehensive data as addressed above.

Especially if the spatio temporal dimension and resulting exposure cannot be sufficiently defined, major uncertainties in the formulation of risk hypothesis are likely to impact the risk assessment process. These uncertainties also will impact case-specific monitoring as well as general surveillance.

In conclusion, the problems of carrying out adequate long term case-specific monitoring and the lack of availability of sufficiently effective methods to prevent and / or mitigate adverse effects in urgent cases, might cause the risk manager to not approve releases of gene drive organisms and other SPAGE.

Whatever the case, the risk manager should be aware of the need to generally restrict the number and scale of releases of SynBio LMOs into the environment in order to prevent unintended transboundary movements. Similar control mechanisms are needed to keep control of potential cumulative adverse effects on health and the environment, and to avoid passing potential tipping points causing irreversible damage to ecosystems. The concepts of nature conservation and environmental protection are largely based on the principle of avoiding interventions. These should also, in particular, be applied in the field of genetic engineering, for SynBio LMOs, gene drives and other SPAGE.

There is increased an awareness that nature and living beings should not only be treated with respect but considered as rights-holders against misuse and destruction (Chapron et al., 2019). If, however, genetically engineered organisms are introduced into natural populations without effective control, this would mean the genetic engineering of the ‘germ line’ of biodiversity, with the risk of disrupting functioning existing ecosystems and their future evolutionary dynamics.

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