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Foreword

The role of science and technology as drivers of change and response mechanisms to some of the global challenges, including those related to biodiversity, cannot be overstated. This perspective is clearly demonstrated when we consider the field of synthetic biology.

Synthetic biology is a rapidly evolving, multidisciplinary field that has generated interest among numerous sectors. It has the potential to bring novel solutions to pressing global issues, such as biodiversity loss, climate change, conservation, hunger and vector borne diseases, among others. Many applications are also emerging to replace industrial processes, for chemical synthesis and to produce new medicines. However, it is important to consider that, as is the case for any technology, the use of synthetic biology may also come with risks, which if not carefully considered, may lead to adverse impacts on biodiversity, ecosystems and the environment. Additional social, ethical and economic considerations have also been raised regarding the use of synthetic biology applications and tools. Thus, we have a responsibility to consider the use of technologies and applications in a manner that promotes safety and incorporates a whole-of-society approach, such that everyone can benefit while minimizing the risks to biodiversity, global health and the environment.

Due to the increasing interest and applicability of synthetic biology, it should come as no surprise that many organizations have also begun exploring how synthetic biology impacts their mandate. Thus, this moment marks a strategic opportunity to foster networking, collaboration and interaction between the organizations and stakeholders within the field, which could help us in moving forward towards a collaborative and non-duplicative approach.

In the context of the Convention on Biological Diversity, a process is under way to negotiate and adopt the post-2020 global biodiversity framework. Within the proposed framework, synthetic biology and its associated technologies can be viewed as part of a larger collection of the tools and solutions to reduce threats and meet people's needs. Promoting and ensuring their safe use will help us achieve the 2050 vision of living in harmony with nature.

The Parties to the Convention have demonstrated interest in the potential impacts of synthetic biology on the three objectives of the Convention, and through decision 14/19, requested the Secretariat of the Convention to update the Technical Series on synthetic biology that was published in 2015. Similarly, a large number of stakeholders have also shown interest by actively participating in the peer review of this document, together with Parties. It is with great pleasure that we present the updated version of the document, which I believe will make an important contribution to the understanding of the complexities of this topic.

I would like to express our gratitude to the International Centre for Genetic Engineering and Biotechnology, for its work on this document along with the Secretariat.

I hope that this document will be used by a wide range of actors and stakeholders and that it will contribute to the global body of knowledge.

Elizabeth Maruma Mrema
Executive Secretary
Convention on Biological Diversity

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Abbreviations and acronyms

3D	Three-dimensional
A, C, G, T	Adenine, cytosine, guanine, thymine
ABS	Access and benefit-sharing
AHTEG	Ad Hoc Technical Expert Group
AIA	Advanced informed agreement
BCH	Biosafety Clearing-House
bp	Base pair
BWC	Biological Weapons Convention
CATCHA	Cas9-triggered chain ablation
CBD	Convention on Biological Diversity
CFS	Cell-free systems
CHACR	Construct hitchhiking on the autocatalytic chain reaction
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CPB	Cartagena Protocol on Biosafety
CRISPR	Clustered regularly interspaced palindromic repeats
DARPA	Defense Advanced Research Projects Agency
DBTL	Design-Build-Test-Learn
DIY Bio	Do-it-yourself biology
DNA	Deoxyribonucleic acid
DSI	Digital sequence information
dsRNA	Double-stranded RNA
e-CHACR	Erasing construct hitchhiking on the autocatalytic chain reaction
ENMOD	Environmental Modification Convention
ERACR	Element reversing the autocatalytic chain reaction
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GATT	General Agreement on Tariffs and Trade
GMO	Genetically modified organism
gRNA	Guide RNA
GURT	Genetic-use restriction technology
HPXV	Horsepox virus
IAS	Invasive alien species
ICSWGSB	International Civil Society Working Group on Synthetic Biology
IGC	Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore
iGEM	International Genetically Engineered Machine
IGSC	International Gene Synthesis Consortium
ILC	International Law Commission
IP	Intellectual property
IPLCs	Indigenous peoples and local communities
IPPC	International Plant Protection Convention
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
IUCN	International Union for Conservation of Nature
LMO	Living modified organism
LMO-FFP	Living modified organism for food, feed and processing

miRNA	MicroRNA
MN	Meganuclease
NASEM	National Academies of Sciences, Engineering, and Medicine
NGO	Non-governmental organization
nt	Nucleotide
ODM	Oligonucleotide-directed mutagenesis
OECD	Organisation for Economic Co-operation and Development
OIE	World Organisation for Animal Health
OPCW	Organization for the Prohibition of Chemical Weapons
PCR	Polymerase chain reaction
PGRFA	Plant genetic resources for food and agriculture
PIP	Pandemic Influenza Preparedness
R&D	Research and development
RdDM	RNA-directed DNA methylation
RIDL	Release of insects with a dominant lethal
RNA	Ribonucleic acid
RNAi	RNA interference
SBSTTA	Subsidiary Body on Scientific, Technical and Technological Advice
SCBD	Secretariat of the Convention on Biological Diversity
SDN	Site-directed nuclease
siRNA	Small interfering RNA
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures
sRNA	Small RNA
SSA	Semi-synthetic artemisinin
SWG	Scientific working group
TRIPS	Trade-Related Aspects of Intellectual Property Rights
TXTL	Transcription-translation
UAA	Unnatural amino acid
UK	United Kingdom of Great Britain and Northern Ireland
UNCLOS	United Nations Convention on the Law of the Sea
UNDRIP	United Nations Declaration on the Rights of Indigenous Peoples
UNESCO	United Nations Educational, Scientific and Cultural Organization
UPOV Convention	International Convention for the Protection of New Varieties of Plants
USA	United States of America
VBD	Vector-borne disease
WHO	World Health Organization
WIPO	World Intellectual Property Organization
WTO	World Trade Organization
XNA	Xenonucleic acid
ZFN	Zinc finger nuclease

Glossary[2]

Artificial intelligence: a field that leverages computers and machines to mimic the problem-solving and decision-making capabilities of the human mind. It combines computer science and robust data sets, to enable problem-solving and seek to create systems which make predictions or classifications based on input data. It also encompasses sub-fields of machine learning and deep learning.

BioBricks: sequences of DNA encoding a biological function that are intended to be modular parts that can be mixed and matched by researchers designing their own devices and systems.

Biofoundries: industrialized, integrated infrastructure for the rapid prototyping and genetic modification of biosystems for a variety of applications.

Bioinformatics: the use of computational technologies to store, mine, retrieve, and analyse data from sequencing technologies by creating unified data models, standardizing data interfaces, developing structured vocabularies, generating new data visualization methods, and capturing detailed metadata that describe various aspects of a biological system.

Cell-free systems: tools consisting of molecular machinery extracted from cells, containing the enzymes necessary for transcription, translation, and other cellular processes independent of a cell.

Directed evolution: a supporting method, process, or technique often employed to engineer improvements in enzymatic performance, consisting of iterative rounds of mutagenesis and screening or selection on the genome scale.

DNA sequencing: the process of determining the nucleic acid sequence or order of the four bases: adenine, guanine, cytosine, and thymine.

DNA synthesis: The chemical or enzymatic synthesis of a DNA molecule of a particular sequence.

Engineered gene drives: genetic elements or constructs that have specifically designed mechanisms that increases the frequency of their inheritance more than expected based on Mendelian inheritance alone.

Genome editing: A suite of tools (oligonucleotide mutagenesis and site-directed nucleases) that can facilitate targeted changes to the genome. CRISPR-Cas is an example of a widely known tool for genome editing.

Genome-level engineering: a field of research that focuses on manipulating or engineering at the whole-genome level of an organism.

Machine learning: a branch of artificial intelligence and computer science which focuses on the use of data and algorithms to imitate the way that humans learn, gradually improving its accuracy.

Metabolic pathway engineering: the intentional modification of cellular metabolism or biochemical pathways for the optimized production or utilization of a particular compound.

Modelling: the mathematical and computational design or representation of an application or system and the analysis of its expected behaviour.

2 This glossary does not intend to provide a definitive definition for each term, but rather to provide clarity and brief information on the terms used frequently within this document. The reader is kindly directed to sections 1 and 2 for a more complete description of some of these terms.

Nucleic acid-based circuits: the rational design of sequences of DNA and RNA to create biological circuits with predictable, discrete functions, which can then be combined in modular fashion in various cell hosts.

Protein engineering: the design of new proteins or modification of the sequence of proteins to create proteins with new, desirable or optimized functions.

Protocells: artificial cells that have some properties of living systems but are not yet fully "alive". A synthetic protocell is likely encoded by a minimal genome, which specifies all essential functions and that allows the protocell to thrive (e.g. coordinated transcription–translation).

RNA interference: an intrinsic cellular mechanism present in almost all eukaryotic organisms and that leads to silencing of gene expression.

RNA-based tools: tools based on RNA biochemistry, such as RNA interference, riboswitches and synthetic small RNA molecules.

Xenobiology: the study of biological systems based on unusual biochemistries derived by chemical compounds of mostly anthropogenic origin and deliberately created in the laboratory. Xenobiology aims to alter the "biochemical building blocks of life" such as by modifying genetic information to produce xenonucleic acids (XNA) or by producing novel proteins containing unnatural amino acids.

Executive summary

Synthetic biology has been described as a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems (SCBD, 2015). As this multidisciplinary area of research continues to advance rapidly, it carries hopes and aspirations to address a multitude of global challenges related to food, health and the environment, but also inspires concerns about potential impacts including those associated to biodiversity.

Synthetic biology relies on a suite of supporting technologies and tools, some of which are also used in the established field of genetic engineering.[3] The more recent emergence of increasingly advanced technologies and tools has greatly expanded the potential range of synthetic biology applications. Consequently, the number of products and potential applications increases and leads to advances in plant and animal engineering, personalized medicine, and clinical therapeutics among others. For example, preliminary research suggests that advances in the application of CRISPR-Cas could help to increase yield and quality and improve disease resistance and stress tolerance of crops, as well as refining breeding methods and supporting accelerated domestication. Moreover, other technologies such as engineered gene drives could now potentially be applied to a variety of organisms as a tool to spread desirable traits throughout a population with a view to fighting vector-borne diseases or protecting biodiversity from invasive species. These tools and areas of research, in conjunction with other methods, have led to a range of potential applications that could be categorized by their intended use in (a) contained, industrial, or laboratory settings, (b) semi-managed, managed, or urban settings, or (c) unmanaged or wild settings. For each of these categories, research and development is ongoing, some products are near-term,[4] and in some cases products have already been released and/or are commercially available.[5]

As synthetic biology moves forward, each development can lead to an array of particular potential impacts, some of which are complex in nature. As a result, there is an acute need to acquire and share knowledge and data to inform the development and implementation of policies. In this context, experience with issues such as handling invasive alien species and the risk assessment and risk management as deployed for early applications of modern biotechnology such as living modified organisms (LMOs) could already provide valuable indications to guide strategic discussions and the analysis of potential impacts for some applications of synthetic biology.

As some synthetic biology applications approach near-term and/or deployment with accompanying potential environmental release, there is a need to decide on whether and how they are to be regulated. Accordingly, there is a need to evaluate their potential impacts. Acknowledging the diversity of potential impacts, these cannot be generalized for all synthetic biology applications, and they should, by necessity, be considered on a case-by-case basis. Definition of the scope of any regulatory approach is complicated by the lack of consensus on what technologies, tools, and applications are or will be considered synthetic biology. For applications deemed to require oversight, a range of approaches may be considered by different jurisdictions. Approaches could include the application or adaptation of existing regulatory regimes for genetic engineering or the establishment of new regimes.

3 "Genetic engineering" is used consistently in this document to mean "the use of molecular biology technology to modify DNA sequence(s) in genomes" (Lanigan et al., 2020).

4 Products that have already undergone a series of regulatory approvals to advance through development, such that the next step is final regulatory approval for access by end-users, for example inclusion in sectorial product registers (e.g. plant varieties, pharmaceuticals, etc.), placing on the market and/or distribution to users.

5 In this document, "commercially available" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

In light of the cross-cutting nature of synthetic biology, the range of actors potentially involved in its governance has grown and is likely to continue to do so. While this ensures a broad collection of perspectives, it will be a challenge to build consensus on the boundaries of the field of synthetic biology.

The governance of synthetic biology is currently supported by a range of international treaties, laws, processes, and initiatives, based on factors such as the products and processes involved, the purpose for which they are applied, and the transboundary implications of their use. However, the current regulatory landscape at the international level is fragmented, creating a complex situation with the potential for regulatory gaps and overlaps, as well as the development of potential synergies and convergence. While enhanced regulatory oversight and/or coordination to address potential gaps and areas of convergence has the potential to strengthen the governance of synthetic biology, there is also a risk of creating an overly complex or stringent environment that slows development in the field.

Science-based risk assessment is a cornerstone of the regulation of technologies. However, due to the diverse nature of the potential impacts, including socioeconomic considerations, in addition to the different views triggered by synthetic biology related to risks and benefits, as well as moral and ethical values, a science-based risk assessment is seen by many as only one element (albeit an important one) of a wider decision-making process. Calls for improved governance of synthetic biology in the international legal and regulatory frameworks, place significant emphasis on the need to better address challenges that go beyond biophysical considerations by considering societal, economic, and ethical dimensions. Further discussion and enhanced regulatory oversight and coordination addressing these dimensions may be desirable to promote public trust and acceptance. However, some of the international treaties, laws, processes and initiatives analysed appear constrained in their ability to address several of these dimensions. In this context, with over a decade of substantive decision-making addressing synthetic biology, the Convention on Biological Diversity has emerged as an important international forum currently deliberating the potential positive and negative impacts of synthetic biology, particularly as they relate to biodiversity and biosafety.

There is a recognized need to first better integrate and coordinate governance of synthetic biology, and secondly, to expand the focus of governance beyond biosafety, human health and the biophysical environment to a more holistic approach that also encompasses social impacts, ethical principles, and elements of social justice, in accordance with national circumstances. As has been recognized before, synthetic biology has the potential to help tackle global challenges such as climate change or biodiversity loss. However, to avoid potential unintended irreversible environmental damage and associated geopolitical challenges, innovative research guidelines, governance methods, integration with social sciences, and engagement with communities need to be strengthened. As synthetic biology advances into the future, the challenge lies in creating a framework that fosters scientific creativity and allows research and product development to move ahead while acting responsibly and in a manner that embraces legal, ethical, and larger societal values.

This publication – an update of CBD Technical Series No. 82 on Synthetic Biology (2015) – attempts to provide an overview of the technical, legal and social issues around synthetic biology and its governance. The key messages from the publication are summarized below.

KEY MESSAGES

The current state of synthetic biology

1. **Synthetic biology is a cross-cutting and rapidly advancing discipline with increasing relevance to the environment, food and health, among other global challenges.**

Synthetic biology relies on a suite of supporting technologies and tools that enable the engineering and creation of biological components. These tools include DNA synthesis, next-generation sequencing, bioinformatics, directed evolution, genome

editing, engineered gene drives, RNA-based tools, modelling, artificial intelligence, machine learning, biofoundries, and BioBricks. Synthetic biology also covers several areas of research such as nucleic acid-based circuits, protein engineering, metabolic pathway engineering, genome level engineering, protocell construction, xenobiology, and cell-free systems. This creates a diverse and rich environment for developing new applications and products. However, the pace of development and application of these technologies and tools vary. The degree of maturity of plant synthetic biology, for example, is lagging behind bacterial, yeast and mammalian systems, where these approaches are already reshaping fundamental research and the biotechnology or biopharmaceuticals industries.

With an expanding diversity of innovative tools, the number of products and potential applications of synthetic biology is increasing and leading to advances in industrial productions, plant and animal engineering, personalized medicine, and clinical therapeutics among others. Applicable in many different sectors, it offers additional prospects for addressing current global challenges and could contribute to achieving diverse United Nations Sustainable Development Goals, including Zero Hunger; Good Health and Well-being; Clean Water and Sanitation; Affordable and Clean Energy; Industry, Innovation and Infrastructure; Responsible Consumption and Production; Climate Action; Life below Water; and Life on Land.

2. Consensus on which techniques or applications fall under the definition of synthetic biology has yet to be achieved.

The Conference of the Parties to the Convention on Biological Diversity in 2016 acknowledged as an operational definition that "*synthetic biology is a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems*". However, there is no internationally agreed definition of "synthetic biology" yet, in part due to the lack of consensus of which techniques or applications could fall under this area. Divergent views in this respect pose challenges at various levels, that range from the governance of synthetic biology to more specific aspects such as problems in applying requirements regarding (future) regulation, among others. However, it should be noted, that even in the absence of an agreed definition, there is interest and will at various levels (national, regional and international) to take advantage of new technologies, including synthetic biology, in a responsible manner that could maximize the potential benefits while minimizing the risks.

3. With early applications reaching large-scale deployment, the economic value of the synthetic biology market continues to increase.

While some research in synthetic biology is focused on elucidating a greater understanding of the essential functions of genomes and the development of non-profit applications, most of the research is focused on commercial and industrial applications. The global synthetic biology market[6] was estimated to be valued at US$ 6.8 billion in 2020 and is projected to grow at a compound annual growth rate of 23.9% during the period 2020-2025. This can be attributed to the rising demand for products and components, especially those produced in containment, e.g. synthetic DNA, synthetic RNA, and oligonucleotides across various industries, as well as the increasing use of engineering technologies for manipulating complex genomes, the increasing development of therapeutics, and the increasing technological advances in the CRISPR toolbox.

6 In the consulted market study, synthetic biology is described as the engineering and design of new biological parts and functions, such as artificial biological pathways and organisms, which can further be used to produce end products, such as biofuels and biosensors, among others. It states also that synthetic biology has a wide range of applications in both the academic and life sciences industries, including biochemicals, pharmaceuticals, drug discovery and therapeutics, and bioremediation. As such, the figures provided would differ if the operational definition of synthetic biology considered in the present document, with the broad range of areas of research, applications, and products, had been considered.

4. **Despite its potential global deployment, research and development in synthetic biology mostly occurs in a limited number of countries.**

By 2016, more than 25,000 authors at 3700 organizations located in 79 countries had contributed to the body of research identifying itself as synthetic biology. Over 13,000 papers on synthetic biology have been published, with the USA, UK, Germany, China, and France leading the number of publications. Other sites of major research include Japan, Switzerland, Italy, Spain, and Canada. Despite the global spread of a reported 2800 research sponsors, there is a concentration among a subset of funding agencies. A cluster of top 20 global sponsors of synthetic biology research were recognized in 70.6% of the synthetic biology articles. This cluster included six funders from the USA, three from China, two from Canada, two from Germany, two from Japan, two from the UK, two from the EU, and one from the Republic of Korea. The top 50 are public research councils or government agencies.

Initiatives designed to facilitate technology transfer, scientific cooperation and capacity-building, cross-border collaboration and access to cutting edge technologies need to be leveraged to enhance research, development and deployment in other countries with limited capacity in synthetic biology. Furthermore, exploring intellectual property options and fostering Open Science to support scientific cooperation and to make science more transparent, accessible, equitable and inclusive, may assist in narrowing the technology divide observed in relation to synthetic biology.

5. **While most applications of synthetic biology so far have been within contained industrial or laboratory settings, this is changing rapidly, with an increasing focus on managed, semi-managed or urban settings, and in the longer term a potential for use in unmanaged and wild settings.**

Synthetic biology provides a useful toolbox for tailoring organisms for new applications and products. So far, most of these applications of synthetic biology engineer microbes to produce alternatives to naturally occurring molecules within contained industrial or laboratory settings, although the range of host organisms is changing rapidly. Currently, two synthetic biology products are commercially available for use in managed settings: one genome-edited crop and one biological nitrogen fertilizer based on engineered bacteria.[7] It is expected that some other genome-edited organisms and potentially also living modified organisms containing engineered gene drives could be deployed and released in the foreseeable future. On the other hand, synthetic biology products intended for unmanaged or wild settings remain in an early stage of research. Nevertheless, synthetic biology applications will no longer be confined to laboratories and contained facilities but will be introduced in environments where interaction between these products and biodiversity will be more likely. Table 1 provides a summary of the synthetic biology applications categorized by their intended use and their level of development.

Potential impacts from synthetic biology

6. **The potential of the synthetic biology toolbox is vast, and so is the potential for impacts on various sectors.**

Synthetic biology's rapid development can be seen in the numerous applications that have reached the market (mainly intended for contained or industrial uses) and those that are near-term products or under research. There are a wide range of synthetic biology products that are currently on the market for various uses such as semi-synthetic artemisinin, squalene, vanillin, shikimic acid, and select fragrances and flavours. Some synthetic biology applications are targeting the replacement of natural materials to take pressure off wild populations, as is the case of the production of recombinant Factor C from synthetic horseshoe crab blood, synthetic rhinoceros horns and squalene, each of which could reduce or remove the need (or perceived need based on unfounded belief) to exploit wild species. Some

7 These products may not be considered as synthetic biology products by all readers in light of the divergence of views held in this regard.

of the applications under research directly target global challenges such as climate change by for instance aiming at increasing the resilience of species to climate change (e.g. in corals), or in designing "next-generation" biofuels. Applications containing engineered gene drives are being designed to modify, suppress or eradicate populations of various target species. Such developments targeting invasive alien species, human disease vectors, agricultural pests and others with conservation objectives are currently at various stages of research and development. These are only some of the many examples of synthetic biology applications that are having, or could have, impacts.

It is worth noting, however, that even though there are numerous synthetic biology applications under research and development, and many that have reached the market, only a few applications developed for direct use in the environment have been authorized to date. This means that there is relatively little field data collected concerning their potential impacts. Therefore, except for a few specific cases where release has been authorized, the range of potential impacts of synthetic biology applications on the conservation and sustainable use of biodiversity remains largely based on theoretical analyses. Thus, the discussions on potential impacts have been informed by limited small-scale experiments mostly in the laboratory and by previous experience with for instance LMOs with similar traits. This brings challenges for achieving consensus on whether and how synthetic biology applications are to be assessed and regulated, and whether any regulation should make use of the same regimes as those in place for LMOs, with or without adaptations, or whether new regimes should be developed.

7. **Many of the assessments of potential impacts that were originally anticipated were overly simplistic in nature; more recent information revealed complexities suggesting the need for a more nuanced approach.**

For example, in some cases, the substitution of natural products (e.g. naturally occurring molecules and compounds obtained from plants) with synthetic analogous products could lessen the pressure on natural habitats and be an effective way to curb illegal wildlife trade. Care should be taken, however, as they may also inadvertently lead to a disruption of conservation efforts, e.g. the substitution could lead not only to an increase in the demand for illegal natural specimens by normalizing use, but also provide cover for mixed shipments with illegal natural specimens. Further, more nuances can derive for example from cases where substituted natural products potentially ease negative pressures on wild or cultivated species, but displace traditional cultivation practices, often in tropical and subtropical regions. If not handled sensibly, this may bring them into conflict with, or displace, those naturally sourced products which underpin the livelihoods and fragile economies of smallholder producers. This complex web of potential interactions, which may not be unique to synthetic biology, is therefore adding to the challenges of assessing *ex ante* the potential impacts that could be associated with its use.

Communication, engagement and transparency

8. **Early society and community engagement in synthetic biology research is a key component of transparency.**

Interest in, and discussions about, synthetic biology are now much more visible and have drawn attention from a wide range of actors such as academia, industry, non-governmental organizations, national Governments, international organizations, and indigenous peoples and local communities (IPLCs), among others. International discussions about synthetic biology currently occur in a wide range of settings and formats. Some examples of activities that stimulate the debate on the matter are the meetings of international organizations, such the Convention on Biological Diversity and other United Nations bodies, other organizations such as non-governmental organizations, industry and academia. The current debate associated with synthetic biology tackles scientific issues, as well as consideration of social, economic, ethical and political aspects. The attention that synthetic biology is receiving, and the engagement of multiple stakeholders in

the debate, opens up opportunities for the sharing of knowledge and information, as well as for cooperation at various levels.

Recognizing the global nature of synthetic biology applications and the fact that local communities are likely to be impacted, it would be advantageous to communicate concepts of new applications prior to large investments of time and resources (e.g. construction, testing and release). Early engagement with potentially impacted communities, including IPLCs, could provide opportunities for discussions related to potential benefits, risks, and concerns. Sufficiently broad and inclusive discussions could in turn improve public trust through the development of safety measures and policies that reflect a broad range of stakeholder views, as well as assisting with the full and effective participation of IPLCs. Further, since most research and development of synthetic biology applications occurs in relatively few countries, outreach and engagement with intended recipient countries and communities will be important when considering release in other geographical locations, especially as there may be a need for further building of local regulatory capabilities.

9. Greater public engagement in regulatory decision-making is needed to ensure that regulatory practice best meets societal expectations/desires/goals.

Regulatory decision-making on activities involving synthetic biology products requires more than just a crucially important assessment of characterized risks and potential prescribed risk management strategies, as the degree to which a risk is acceptable is a social construct, as are the guiding policy goals. Neither can be determined purely scientifically. Therefore, decision-making should be informed through a scientifically sound risk assessment and consultation with a broad set of stakeholders, including, among others, any communities of people likely to be impacted the most as well as experts in the field. For emerging technologies that may affect the global commons, there has been a call for concepts and applications to be published in advance of construction, testing, and release. This lead time would enable a public discussion of environmental and security concerns and needs, research into areas of uncertainty, and the development and testing of biosafety features. It would also allow any necessary adaptation of regulations in light of emerging information on benefits, risks and policy gaps. Even more importantly, it would allow broadly inclusive and well-informed public discussion to determine if, when, and how some applications should be used. The importance of participatory decision-making and public acceptance is increasingly being recognized, particularly related to the free, prior and informed consent (FPIC) of communities, including IPLCs, concerning the environmental release of engineered gene drives.

10. Biosecurity risks arising from synthetic biology could be mitigated through improved transparency, communication and self-regulation.

"Dual use" refers to the possible misuse of research with legitimate scientific purpose to pose a threat to public health and safety, agriculture, the environment and/or national security. It is an important concern raised by the life sciences including those underpinning synthetic biology. If not appropriately addressed, it threatens to undermine public confidence. Government defence funding of some leading-edge synthetic biology projects have triggered concerns regarding appropriate oversight and use; however, any non-pacific uses arising from such applications would place the sponsor in violation of the Biological Weapons Convention. The "DIY Bio" community has also triggered similar concerns, and although they have been proactive about biosecurity and biosecurity education, concerns persist as to what amateurs working in synthetic biology may be able to do in low tech laboratory settings. One way to reduce biosecurity concerns in synthetic biology is to make sure that biosecurity is given more prominent support and that research into improvements in biosecurity is specifically funded. Another is for scientists to appropriately communicate work that may lower barriers to biological weapons development. Researchers working on projects with potential dual-use applications need to communicate that the potential risks have been

carefully considered, and policymakers need to consider whether the research is sufficiently important to pursue in the public interest. Additionally, self-regulation may also play an important role in assuring biosecurity, such as the screening protocols implemented by DNA synthesis companies to prevent orders of potentially dangerous genetic materials, as well as through approaches promoting the evaluation of societal implications of emerging technologies and improved governance concerning transparency, accountability, integrity, and capacity (for example, promoting Responsible Research and Innovation, Governance Coordinating Committees and comparable approaches).

Synthetic biology regulation and governance

11. For synthetic biology to live up to its perceived potential, an enabling policy and regulatory environment is needed.

Many regulatory mechanisms were developed before some of the tools that enable synthetic biology, and even before the term synthetic biology became widely used. As synthetic biology advances, regulatory mechanisms may need updating on a case-by-case basis to address some of its applications. It is therefore imperative that resources be available for the development and/or adaptation as needed, of regulatory systems that could address the different aspects related to the specific applications of synthetic biology, including the furtherance of the objectives of the Convention on Biological Diversity.

The cross-cutting nature of synthetic biology, although not unique to this area of research, may bring challenges not only under the Convention and its Protocols, but for other regulatory frameworks which are relevant to its international governance, including those governing public health, conservation, commerce and trade, risk of harm, FPIC, and access and benefit-sharing.

In the context of the Cartagena Protocol on Biosafety, for instance, many developments in synthetic biology will put even greater pressure on those developing countries that have not yet developed national biosafety regulatory frameworks. These systems will have to be developed or adapted to accommodate the potential expansion of the range of LMOs and applications. Such adaptation may require a concerted effort from all stakeholders to "future-proof" existing frameworks as necessary, noting of course asymmetries in the resources of countries and stakeholders and their capacities to adapt. Frameworks and regulatory approaches which use a general-purpose criterion may facilitate adaptation regardless of future scientific and technological developments in synthetic biology.

12. International governance and regulation associated with synthetic biology is complex and would benefit from a coordinated and cooperative approach.

Considering the broad scope of synthetic biology research, as well as the potential positive and negative impacts of its products and applications, it is not surprising that no international treaty framework nor institution exists with a sufficient mandate to regulate the full spectrum of possible synthetic biology activities or products. The multifaceted landscape of international instruments, regimes and initiatives has potential implications for the governance of synthetic biology products and applications. This international patchwork spans biodiversity, biosafety, biosecurity, health, FPIC, access and benefit-sharing, intellectual property, commerce and trade among others. Given the diverging mandates and priorities across international fora, it will be beneficial for the international organizations with overlapping or complementary mandates to collaborate, for example, in balancing risks and potential benefits arising from specific synthetic biology applications, in a holistic and integrated manner.

The Convention on Biological Diversity is the primary forum deliberating the governance of synthetic biology applications and products in relation to potential impacts on biodiversity-related issues. The framework of the Convention also provides unique opportunities for hosting discussions aimed at improving coordination and addressing challenges and opportunities for cooperation which

are apparent in the governance of synthetic biology, while respecting the competencies of other international fora where overlaps exist.

Engagement with consortia and multi-stakeholder initiatives focused on synthetic biology could play a key role in facilitating coordination among government, academia, and industry and the development and adoption of norms. In addition, the breadth of the cross-cutting and multidisciplinary natures of synthetic biology is an important factor to consider in any potential scenario towards its governance and regulation. It is unlikely that a single entity will have the necessary mandate, capacity, knowledge, and tools to have a meaningful impact alone. Coordination, cooperation, capacity-building, knowledge-sharing, technology transfer and communication are of paramount importance, particularly as they relate to the development of best practices and shared principles across international fora. The post-2020 global biodiversity framework and its long-term strategic framework for capacity-building, in particular, will provide opportunities for coordination across international fora and an opportunity to minimize the technology and capacity gaps between developed and developing countries in relation to synthetic biology. The governance of synthetic biology cannot advance successfully if the approach towards it is narrow or if it lacks the support of the various entities and stakeholders who play a key role in its development, dissemination, potential regulation and potential use.

13. **Better understanding on what to expect in terms of developments will play a key role in helping regulatory systems to keep up with the fast pace of development of synthetic biology.**

Considering the fast pace of development of synthetic biology, and the challenge for regulatory regimes to cope with potential new applications, an early screening of what is under research and development and their commercialization perspectives will be important to consider in order to provide timely information for countries and organizations to react and adapt if necessary. This would be assisted by open dialogue between the synthetic biology community and regulatory bodies to help prepare each of them to address any concerns from society. Regulators should also supplement these conversations with regular independent horizon scanning or foresight studies with adequate public and stakeholder engagement in order to enhance transparency, build trust and capitalize on various sources of expertise.

Table 1. Applications of synthetic biology categorized by their intended use and degree of development. See section A ("Scope and methods") for rationale concerning inclusion of products under synthetic biology and section 3 for further details regarding synthetic biology applications

Intended use	Research	Near-term products[8]	Commercially available[9]
Containment, industrial processes, or laboratory settings	• Development of protocells and minimal cells for basic research • Applications to produce non-native nucleotides and amino acids inside the cell for basic research and production of pharmaceuticals • Re-creation of extinct virus from chemically synthesized DNA • Re-creation of an extinct infectious horsepox virus from chemically synthesized DNA fragments • Genetically engineered biocontainment systems within the cell • Digital information storage using DNA molecules • Synthetic biosensing circuits and biosensors	• Engineered algae as biofactories for chemicals or renewable fuel • Biofabricated wildlife products • Cultured leather products • Plant-based vaccines • Engineered phages as antimicrobials • Engineered probiotics for the production and *in vivo* delivery of medicines • Food and food ingredients	• Biopharmaceuticals • Carbon recycling • Fabric • Cosmetics and fragrances • Food and food ingredients • Part, devices, and systems
Semi-managed, managed, or urban settings	• Engineered gene drive for an agricultural pest • Genetically engineered bacteria for environmental applications, such as bioremediation, biodegradation and biomining • Genetically engineered nitrogen-fixing bacteria and other genetically engineered bacteria for agriculture • Virus-induced genome editing • Projects for the de-extinction of extinct animals • Transient modification of agricultural plants through RNAi spray or nanomaterials • Genetically engineered plants to produce recombinant polyclonal antibodies against snake venom toxins	• Self-limiting insects • Genome-edited crop plants and farm animals • Engineered gene drives in mosquitoes for control of vector-borne diseases[10] • Genetically engineered sorghum to produce a new synthetic protein to improve the digestibility in food and feed • Genetically engineered oilseed rape to enhance resource use efficiency of existing cropland	• Genome-edited soya bean • Biological nitrogen fertilizer based on engineered bacteria

8 Products that have already undergone a series of regulatory approvals to advance through development, such that the next step is final regulatory approval for access by end-users, for example inclusion in sectorial product registers (e.g. plant varieties, pharmaceuticals, etc.), placing on the market and/or distribution to users.

9 In this document, "commercially available" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

10 This application could also be considered under the category of unmanaged or wild settings, depending on how and where it is used.

Intended use	Research	Near-term products[8]	Commercially available[9]
Unmanaged or wild settings	• Engineered gene drive applications for control of invasive rodents • Engineered gene drives to control vector-borne diseases (e.g. schistosomiasis) • Genome-edited coral for climatic resilience • Genome-edited mice to prevent transmission of Lyme disease • Genome-edited amphibians for resistance to fungal pathogens	• No information[11]	• No information

11 Engineered gene drives in mosquito for potential control of vector-borne diseases described under category semi-managed, managed or urban settings could also be considered to fit under this category depending on how and where they are used.

A. Scope and Methods

Synthetic biology falls within the scope of biotechnology as defined by the Convention on Biological Diversity (United Nations, 1992), i.e. "*... any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use*". It is also recognized that synthetic biology methodologies and techniques share various degrees of overlap with those of "modern biotechnology" and, in particular, the "*application of in vitro nucleic acid techniques … that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection*" as defined in the Cartagena Protocol on Biosafety (SCBD, 2000).

While acknowledging that there is no internationally agreed definition of "synthetic biology", for the purposes of this document, the authors have used the operational definition of the Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology, that "*synthetic biology is a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems*", which was considered useful as a starting point for the purpose of facilitating scientific and technical deliberations under the Convention and its Protocols in decision XIII/17. It must be underlined that the authors are not championing this to be the definitive definition.

Further, it is also acknowledged that until consensus is achieved concerning which techniques, processes or products will remain under the definition of genetic engineering[12] and those that will now fall under synthetic biology, there will always be a divergence of views and opinions on this among the readers. The authors recognize therefore that a "blurring of the lines" between the two may occur at times; however, it is not the place of this document again to champion any particular distinction between them. Thus, it is expected that there will be different views not only concerning which techniques fall under the above definition of synthetic biology, but also that some readers may not consider some of the processes or products described in this document to be synthetic biology approaches and applications at all. The authors have also attempted to achieve the same degree of inclusivity when presenting the numerous published perspectives concerning individual synthetic biology applications and the research area as a whole, especially when in these publications the individual applications had been identified as synthetic biology in nature. The authors also recognize that the potential benefits and/or risks arising from synthetic biology applications could also be identical or very similar to those from practices that do not involve synthetic biology at all, regardless of which interpretation of the definition is used.

This document has been prepared pursuant to a request from the Conference of the Parties to the Convention on Biological Diversity. By its decision 14/19 (paragraph 17 (c)), the Conference of the Parties requested the Executive Secretary to update an earlier publication – CBD Technical Series No. 82 on Synthetic Biology – originally published in 2015. To remain true to decision 14/19, whenever possible the original structure and outline of the 2015 edition was maintained and updated, instead of creating a completely different document. It is, however, important to note that during the updating, in order to adequately cover the magnitude of recent changes in the field of synthetic biology such that these are the main focus of the document, not only was information added, but tracts of original text from the 2015 edition were also condensed or removed if now outdated or surpassed by more recent developments or information. The document

12 "Genetic engineering" is used consistently in this document to mean "*the use of molecular biology technology to modify DNA sequence(s) in genomes*" (Lanigan et al., 2020).

was also subject to a peer-review process,[13] which triggered further changes on various fronts.

Understanding that there had been an explosion of activity that had occurred in the field of "synthetic biology" in the last five years, the search for relevant published literature was cast as wide as possible. The first tranche of information to update the document was taken from the list of bibliographic references issued by the Secretariat as an information document for the twenty-fourth meeting of the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA).[14] This list of references was then updated during the last quarter of 2020 and the first quarter of 2021, primarily through a bibliographic search of databases of both peer-review literature and "grey literature" published since 2015, i.e. literature produced at all levels of government, academia, industry and civil society, in print and electronic formats. The list of references was further extended to include those necessary to respond to comments received during the peer-review process. Of primacy among the grey literature were documents such as reports of the AHTEGs and decisions of the Conference of the Parties to the Convention and the Conference of the Parties serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety, as well as publications, guidance, and opinions from regulatory authorities. Together, these were supplemented with information from websites and publications of other organizations, especially those entities mandated to implement the international treaties, laws, processes and initiatives listed in section 9 and those civil society organizations with a prominent focus on synthetic biology impacts on the conservation and sustainable use of biological diversity. Specific elements concerning indigenous peoples and local communities (IPLCs), including arguments representing IPLC views, were mainly sourced from AHTEG reports and other Convention documents.

It should also be understood that the coverage of information used for the update is not exhaustive. Further, only a small proportion of the available publications and information analysed the existing international legal and regulatory frameworks, as well as the extent of potential impacts on biodiversity, specifically through the lens of the synthetic biology sector as a whole. The authors have therefore attempted to exemplify the discussed topics with actual examples. When this was not possible, sections of more generalized text have resulted.

The update of the 2015 edition has therefore been made on the basis of the above principles and bibliographic searches. In addition, section B below ("Technical background on synthetic biology") has been updated considering the importance of differentiating between the various stages of applications and products of synthetic biology in the development pipeline, from research through to commercialization,[15] and is also anchored on the rationale used by the Synthetic Biology AHTEG in its report from 2019 (SCBD, 2019). For section C below ("Potential impacts of components, organisms and products resulting from synthetic biology"), the update was made considering that impacts cannot be generalized, and therefore, they should be considered on a case-by-case basis and linked to specific uses. In this light, the section was drafted considering specific examples of synthetic biology applications whenever possible. In doing so, the authors prioritized examples from applications that are either in advance stages of research, are commercialized, or have received significant international attention either through publications and literature or through discussions at international fora (i.e. Convention and other international meetings). It is therefore important to note that the section is not meant to be an exhaustive list of potential impacts for every application of synthetic biology. The update of the section was also done considering that potential impacts could either be directly related to the conservation and sustainable use of biological diversity and/or to socioeconomic and cultural considerations. Section D below ("Synthetic

13 Notification Ref. No. SCBD/CPU/DC/WM/MAQ/MW/89581.
14 CBD/SBSTTA/24/INF/6.
15 In this document, "commercialization" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

biology governance and regulatory perspectives") was updated acknowledging the many international organizations and initiatives, including the Convention and its Protocols, that are discussing synthetic biology. To this end, again, the update is not meant to be an exhaustive list of initiatives or organizations that are engaged in discussions on synthetic biology, or which have programmes of work which consider aspects related to synthetic biology. In doing so, the update prioritized the inclusion of information on international initiatives and did not consider those too many to mention of a more regional or national nature.

B. Technical Background on Synthetic Biology

1. Supporting technologies and tools

Synthetic biology relies on a suite of supporting technologies and tools, some also used in genetic engineering,[16] that have become dramatically faster and less expensive since the 1990s (El Karoui et al., 2019; Royal Academy of Engineering, 2009). The ability to sequence DNA is key to all areas of synthetic biology research. Scientists have been able to sequence and analyse DNA since the 1970s, but high-throughput next-generation sequencing (NGS) methods and computer programmes make it possible to read DNA at more rapid speeds for less money (Royal Academy of Engineering, 2009). The massive amount of data generated by NGS can be integrated using computational tools such as bioinformatics. Together, NGS and bioinformatics facilitate the synthetic biology engineering approach. On the other hand, computational modelling has catalysed synthetic biology research by making both simulation and, to a more limited extent, *in silico* predictions possible (Esvelt & Wang, 2013).

By 2016, more than 25,000 authors at 3700 organizations located in 79 countries had contributed to the body of research identifying itself as synthetic biology (Shapira et al., 2017). According to French (2019), over 13,000 papers on synthetic biology have been published, with the USA, UK, Germany, China, and France leading the number of publications. Other sites of major research include Japan, Switzerland, Italy, Spain, and Canada (French, 2019). Shapira et al. (2017) reported 2800 sponsors of such global research. Despite the global spread of research sponsors, the authors reported a concentration among a subset of funding agencies. A cluster of top 20 global sponsors of synthetic biology research were recognized in 70.6% of the synthetic biology articles. This cluster included six funders from the USA, three from China, two from Canada, two from Germany, two from Japan, two from the UK, two from European agencies and one from the Republic of Korea. The great majority of funders in the top 50 are public research councils or government agencies (Shapira et al., 2017). Fuelled by increasing R&D activities, the synthetic biology market has experienced significant growth in the past decade (Meng & Ellis, 2020). The global synthetic biology market was estimated to be valued at US$ 6.8 billion in 2020 and is projected to grow at a compound annual growth rate of 23.9% during the period from 2020 to 2025.[17] It is important to note that some synthetic biology products are intended for distribution through non-commercial channels, especially those that are being developed with a view to improving public health and biodiversity conservation.

Similar to the divergent views on what is considered synthetic biology, there are also different views on what could be considered a supporting technology or tool. This section provides information on some of the more widely used tools that have been referred to as synthetic biology or related to synthetic biology. Further, it should be noted that they are not exclusively used for synthetic biology but also for other biotechnology processes. The following technologies and tools are not meant to be an exhaustive list, and their use will not necessarily always result in a synthetic biology product, organism or component.

1.1. Synthesis of DNA

The ability to chemically synthesize DNA dates to the early 1970s when a 77 base pair (bp) double-stranded DNA was successfully synthesized

16 In addition to rDNA techniques, synthetic biology uses standardized parts, mathematical modelling, analysis, and computer sciences, *inter alia*, to create novel biological parts or organisms.

17 MarketsandMarkets™ report elaborated by Area Science Park, Italy, for this Technical Series update (22 February 2021). This market report did not consider proteins or enzymes produced in industrial settings.

(Wang et al., 2018). The introduction of automated DNA synthesis machines has saved time and effort on the part of researchers using synthesized DNA for experiments (Garfinkel & Friedman, 2010; Schmidt, 2009). Using appropriate techniques, machines can also create DNA strands up to the size of a gene. Techniques for DNA assembly have also advanced, with laboratories having developed various *in vivo* assembly systems by which bacterial genome-length DNA strands can be assembled at once within a cell (Hughes & Ellington, 2017). It is widely anticipated that tools for DNA synthesis will continue to dramatically drop in price and expand the size and reliability of production (Smanski et al., 2016). The drop in cost for gene synthesis can mostly be attributed to new methods for printing thousands of oligonucleotides in parallel on chips and teaming this with next-generation sequencing (Meng & Ellis, 2020). DNA synthesis is an expanding commercial field, with techniques developing rapidly and new companies being created.

Synthetic biology researchers and innovators depend upon DNA manufactured outside the cell using a technique known as phosphoramidite synthesis. This process, developed in 1981, entails multiple rounds of the stepwise assembly of chemically modified nucleotides (Wang et al., 2018). In the early days, molecular biology relied primarily on short DNA sequences, such as polymerase chain reaction (PCR) primers or probes for molecular detection applications. Now, researchers can use synthesized DNA to assemble entire genes and even synthetic genomes (Hughes & Ellington, 2017). However, such lengthy sequences are not possible by relying on the phosphoramidite process, where the efficiency of direct synthesis steadily drops with the DNA molecule length. Oligonucleotides 60-100 bp in length can be easily synthesised, but for fragments ranging from 200-2000 bp, shorter oligonucleotides need to be assembled. For larger DNA assembly, cloning and enzymatic methods are usually employed (Gibson et al., 2009). Several start-up companies are now pursuing the potential of enzymatic synthesis as a faster – even within a single day – and more efficient route to synthesize longer DNA sequences than is possible with traditional chemical means (Eisenstein, 2020). Moreover, it is expected that in two or three years, the first benchtop DNA printer powered by enzymatic synthesis will be commercially available.[18]

1.2. Next-generation sequencing and bioinformatics

DNA sequencing has revolutionized the fields of biological sciences and allowed for the scientists to begin to investigate the genomic sequence of organisms. More commonly, sequencing technologies are being utilized for whole genome sequencing, metagenomics, RNA sequencing and epigenomics (Buermans & den Dunnen, 2014; Rhoads & Au, 2015). Early sequencing technologies relied on the use of chain-terminating nucleotides during DNA extension to produce fragments of varying lengths (Sanger sequencing) or the chemical cleavage of terminally labelled DNA-restriction fragments (Maxam-Gilbert sequencing). Both methodologies relied on the use of gel electrophoresis to separate the fragments based on size and then X-ray film visualization to allow base-by-base "reading" of the particular sequence. However, these methodologies can only be used for sequences of up to roughly 1000 to 1200 bases in size (Bruijns et al., 2018; Shendure et al., 2017). Developments such as Shotgun sequencing[19] further improved the ability to sequence larger sequences by breaking them up into smaller fragments. However, with the invention of next-generation sequencing technologies, such as pyrosequencing, sequencing by reversible chain terminators, sequencing by ligation and ion torrent sequencing, the ability to sequence much larger sequences has greatly increased. The protocols rely on the preparation of libraries (fragmenting sequences and adding adapters), followed by amplification of the prepared sequences, before sequencing a large number of fragments simultaneously (multiplexing). The key differences from

18 Press release: https://www.dnascript.com/press-releases/dna-script-announces-the-commercial-launch-of-the-syntax-system-the-first-benchtop-dna-printer-powered-by-enzymatic-synthesis-to-accelerate-molecular-biology-and-genomics-workflows/.

19 Shotgun sequencing involves the fragmentation of DNA into small fragments and cloning into a host bacterium. The fragments are then sequenced and the sequence is reassembled based on overlaps between the fragments (Eisen, 2007).

"first generation" sequencing were this ability to multiplex reactions, removal of the need for gel electrophoresis, and creation of libraries *in vitro* instead of in bacteria (Bruijns et al., 2018; Shendure et al., 2017). Recently, new sequencing technologies, such as nanopore sequencing and zero-mode waveguide sequencing, aim to sequence longer lengths without the need for amplification steps while improving the coverage of the sequencing potentially at a real-time resolution of a single molecule. These could improve on previous next-generation sequencing technologies by circumventing the amplification step, which can introduce errors, sequence-dependent biases and add time to protocols (Bruijns et al., 2018; Shendure et al., 2017). Overall, the development of next generation sequencing has precipitated a great reduction in the cost of performing sequencing experiments, a trend that is expected to continue as these technologies are further fine-tuned and developed (Li et al., 2019; National Human Genome Research Institute, 2020).

With the advent of next-generation sequencing technologies, researchers now have the ability to generate over a terabase (10^{12}) of sequence during one run. However, most of the generated sequence is produced in the form of "reads", which can be up to a few hundred bases in length (depending on the protocol). Yet manual "reading" of potentially up to a billion short sequences is not reasonable. The concurrent development of bioinformatic tools has enabled the computational processing of the sequencing data and (re)assembly of the sequences (Shendure et al., 2017). Bioinformatic tools[20] also allow for comparative analyses between data sets and querying of the ever-growing sequence databases (Gauthier et al., 2019). As such, these tools aid in the annotation of putative or novel coding sequences, the development of molecular markers, the exploration of transcriptomic changes under various conditions, the describing of small RNA populations in a sample, the profiling of a proteome of a particular tissue and the uncovering of differential methylation patterns, among others (Chenarani et al., 2021; Di Silvestre et al., 2018; Hu et al., 2018; Westermann et al., 2017; Zanini et al., 2018).

1.3. Directed evolution

Directed evolution is a supporting method, process, or technique often employed to engineer improvements in enzymatic performance, consisting of iterative rounds of mutagenesis and screening or selection on the genome scale (Singh & Braddick, 2015). Researchers create a range of variations in a biological entity and apply selective pressure to them with the goal of identifying those with desired properties. For example, directed evolution of enzymes has tailored biocatalysts for applications, expanding the repertoire of enzymatic activities (Tamaki, 2020). This can be done in two ways: random and targeted (Cao et al., 2020). Various tools can be used to create the variations. For random directed genome evolution, the tools used are mainly related to growth conditions and tolerance to chemicals. For targeted directed genome evolution, some of the current tools used are based on oligonucleotides, RNA, clustered regularly interspaced short palindromic repeats (CRISPR), and recombinases (Cao et al., 2020). A technology called multiplex automated genome engineering, developed by Wang et al. (2009), constituted an efficient tool for genome modifications simultaneously in multiple loci. It was implemented in a range of applications in bacteria, such as changing the genetic code, or the incorporation of non-standard amino acids (Singh & Braddick, 2015). It was also adapted to be applied in eukaryotic cells such as yeast (Barbieri et al., 2017) or mammalian cells (English et al., 2019); however, several other less expensive and less laborious strategies, such as genome editing, were subsequently developed.

1.4. Genome editing

Synthetic biology also employs techniques for genome editing. The term "genome editing" is most often used as if it were a single technology, but it is more accurate to consider each as a suite

20 Bioinformatics is a discipline that uses computational technologies to store, mine, retrieve, and analyse data from sequencing technologies by creating unified data models, standardizing data interfaces, developing structured vocabularies, generating new data visualization methods, and capturing detailed metadata that describe various aspects of a biological system (Fenstermacher, 2005).

of approaches that can be tailored to the needs of specific applications. Genome editing is based on oligonucleotide-directed mutagenesis (ODM) and variants of site-directed nuclease (SDN) technologies. Chemically synthesized oligonucleotides can be used as a template for making targeted genome changes (Sauer et al., 2016). This technique, ODM, has been successfully used to edit genes in bacteria (Drufva et al., 2020; Swingle et al., 2010), in plants to induce herbicide tolerance (Dong et al., 2006; Ricroch & Hénard-Damave, 2016), and in mammalian cells (Aarts & te Riele, 2010; Strouse et al., 2015).

SDN techniques include the use of meganucleases (MN), zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and CRISPR-Cas9 (or alike). These site-directed nucleases can be engineered to bind to DNA sequences in specific manners (Carroll, 2013; Gaj et al., 2016; Lienert et al., 2014). Approaches using SDNs and ODM are applied to introduce random (SDN-1), directed or pre-designed sequence changes (SDN-2 and ODM) at specific, predefined genomic loci. These approaches mostly but not always require the introduction of recombinant constructs into the host organism genome. ODM for example is directed by small-sized synthetic oligonucleotides, which are transiently introduced into the recipient cells and subsequently degraded by the cellular metabolism. SDNs which facilitate genome editing can either be inserted into the genome of the target cell as a transgene or introduced into target cells as functional (ribonucleo)proteins from transiently introduced DNA constructs. Unlike CRISPR-Cas, which relies on DNA-RNA interaction as the mechanism of target DNA recognition, TALEN relies on DNA-protein interactions (Gaj et al., 2016; Khalil, 2020). Some approaches for genome editing, commonly referred to as SDN-3, facilitate the insertion of transgenic constructs at specific genomic locations. The respective changes and transgenic insertions present in the final host organism are heritable (Eckerstorfer et al., 2019). ZFN, TALEN, and CRISPR have been used *ex vivo* to edit prokaryotic and eukaryotic cells (Grohmann et al., 2019). TALENs has been used to efficiently modify plant genomes (Zhang et al., 2013), for example, to create a mutation in rice aiming at increasing its resistance to the bacterial pathogen *Xanthomonas oryzae* (Li et al., 2012), a soya bean with better oil quality (Haun et al., 2014), or a potato with reduced levels of post-cook acrylamide (Clasen et al., 2016).

An enormous increase of studies performing genome editing in crops has been reported, especially the CRISPR-Cas and related systems which have been used lately for almost all genome editing studies (Zhu et al., 2020) and a rising number of market-oriented traits are being investigated or addressed by genome editing research (Menz et al., 2020). In less than a decade, the number of potential applications in crop improvement and breeding of this powerful technology has increased exponentially worldwide, often as an aid to understand gene functions and related traits, as well as to improve the methodologies, increase efficiency and reduce off-target effects (Eckerstorfer et al., 2019). This has led to preliminary research advances in plant and animal genetic studies and engineering, personalized medicine, and clinical therapeutics. Notably, the 2020 Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer A. Doudna, developers of this tool (Uyhazi & Bennett, 2021). Additionally, it is expected that advances in the CRISPR toolbox will register a compound annual growth rate of 18% in the period 2021-2025.[21]

CRISPR-Cas technology is being applied with the aims of increasing plant yield, quality, disease resistance and herbicide resistance, breeding and accelerated domestication (Zhu et al., 2020). For instance, research suggests that it could be possible to efficiently combine agronomically desirable traits with useful traits present in wild counterparts for *de novo* domestication of plants, such as wild tomato (Zsögön et al., 2018) or groundcherry (Lemmon et al., 2018), using genome editing; or to breed plants to adapt them for urban agriculture uses (Kwon et al., 2020). CRISPR tools can also facilitate the control of plant chromosomal recombination (Taagen et

21 https://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=18727.

al., 2020), thereby unlocking otherwise inaccessible genetic diversity. The next generation of CRISPR-Cas technologies and applications are already under way (Adli, 2018; Pickar-Oliver & Gersbach, 2019), and coupled with other approaches, e.g. haploid induction or developmental regulators, it could lead to a new generation of improved crops (Kelliher et al., 2019; Maher et al., 2020). Even though a multitude of traits have been edited, only a few CRISPR-based genome-edited plants have reached the market so far (see section 3), but more crops are in the development pipelines of various companies and may soon appear commercially (Menz et al., 2020; Park et al., 2019).

Base editing is a newer genome editing approach that uses components from CRISPR systems together with other enzymes (Rees & Liu, 2018). This technique installs targeted point mutations without requiring double-stranded breaks or donor DNA templates, and without reliance on homology-directed repair. Two main classes of base editors have been developed to date: cytosine base editors, which catalyse the conversion of C/G base pairs to T/A base pairs; and adenine base editors, which catalyse A/T-to-G/C conversions (Anzalone et al., 2020). Base editors have been applied in a variety of cell types and organisms, including animal models of human genetic diseases, to insert or revert transition point mutations (Anzalone et al., 2020). In addition, base editors originating from bacterial toxins have been applied to edit the genome of organelles, such as mitochondria, in a CRISPR-free manner (Mok et al., 2020).

1.5. Engineered gene drives

A gene drive is a process of biased inheritance from one generation to the next that allows a genetic element (natural or synthetic) to spread rapidly through populations, even in the presence of some fitness cost (Burt & Crisanti, 2018; Alphey et al., 2020). Transposable elements, meiotic drivers, and homing endonuclease genes are examples of natural gene drive systems (Sinkins & Gould, 2006). The potential application of these natural gene drive systems to suppress populations of insects has been studied in field trials since the 1960s. However, in recent years, engineered gene drives have gained in prominence. Like the term "synthetic biology" under which it may fall, the term "gene drive" is most often used as if it were a single technology, but it is more accurate to consider each as a suite of approaches that can be tailored to the needs of specific applications. According to Alphey et al. (2020), the lack of a common definition poses a practical dilemma to researchers, policymakers, and other stakeholders.

Different mechanisms have been developed, but essentially engineered gene drives are genetic elements that are inherited more frequently than expected based on Mendelian inheritance alone. Usually, there is a 50% chance that a genetic element is present in the offspring of sexually reproducing organisms. Each engineered gene drive, however, has a specific designed mechanism that increases the frequency of their inheritance (Champer et al., 2016; Hay et al., 2021; Raban et al., 2020; López Del Amo et al., 2020). Currently, there is an increased interest in gene drives because of the possibilities they offer when combined with the CRISPR genome editing technique, to the extent that these CRISPR-based engineered gene drives may potentially be applied to a wide variety of organisms (Rode et al., 2020). Laboratory-based testing indicates that these CRISPR gene drives are efficient tools to spread traits through a population: an individual carrying a CRISPR gene drive is intended to produce offspring that potentially all carry the gene drive (Rüdelsheim & Smets, 2018).

Among the various engineered gene drives being developed (for updated examples of engineered gene drive approaches, see EFSA GMO Panel (2020) and WHO (2021), CRISPR-based homing gene drives are the most adaptable to new species and populations. Thus, there has been an increase in technological developments with respect to these engineered gene drives (Raban et al., 2020). Homing endonuclease genes are selfish genetic elements that spread horizontally within a host population by first cleaving chromosomes that do not contain them and then being copied across to the broken chromosome as a by-product of the repair process (Burt & Koufopanou, 2004). They involve a piece of DNA

that includes a guide RNA (gRNA) gene and a *cas9* gene (encoding the Cas9 endonuclease). The gRNA is designed to recognize a specific sequence in a wild-type chromosome, so that in heterozygotes carrying a drive allele and a wild-type allele, the Cas9-gRNA molecular complex will cut the wild-type chromosome at the target site. The resulting double-strand DNA break can then be repaired through homology-directed repair (also known as "gene conversion"), using the drive allele as a template, which is designed to harbour sequences identical to the ones flanking the target site. Consequently, the drive allele is transmitted to the next generation at rates beyond those of regular Mendelian inheritance and, if its features allow it, will spread within the target population (Rode et al., 2020).

1.6. RNA-based tools

RNA interference (RNAi) is an intrinsic cellular mechanism present in almost all eukaryotic organisms and leads to silencing of gene expression. Its discovery and description in *Caenorhabditis elegans* by Andrew Fire and Craig Mello led to the 2006 Nobel Prize in Medicine (Nobel Media AB, 2021). The mechanism is triggered by the recognition of double-stranded structures in RNA molecules (Torres-Martínez & Ruiz-Vázquez, 2017). The cellular machinery processes the RNA into small RNA (sRNA)[22] molecules, which then act as a guide template to target RNA sequences with complementarity within the host cell. Upon binding a sequence, silencing is achieved through the degradation of the messenger RNA (mRNA), removal of the polyadenylated tail, blocking ribosomal protein synthesis and/or epigenetic transcriptional repression. In nature, RNAi protects cells from double-stranded RNA (dsRNA) viruses, suppresses transposons and allows for "fine-tuning" of gene expression through the endogenous expression of hairpin-structured microRNA (miRNA) (Duempelmann et al., 2020; Liu et al., 2020; Zotti et al., 2018).

With this understanding, the mechanism can be exploited using RNA expression constructs (encoding antisense, dsRNA or hairpin RNA) delivered to plants as transgenes, as a part of viral vectors or as topical dsRNA sprays (Liu et al., 2020). Thus, these methodologies are being intensively investigated to provide crop protection against arthropod pests, nematodes (Zotti et al., 2018), viruses (Taliansky et al., 2021) and fungi (Fletcher et al., 2020; Machado et al., 2018), as well as to improve nutritional content (Mezzetti et al., 2020). Due to the sequence-specific mode of action, molecules can be designed to target the expression of a single gene, gene family or multiple genes in parallel. Additionally, molecules could theoretically be designed to be species-specific or to have a broader action spectrum, as well as selected to provide various outcomes ranging from sublethal to lethal effects, all depending on the sequence chosen (Cagliari et al., 2019; Taning et al., 2020). The increasingly available *in silico* tools and genomic sequence data sets have facilitated the design of more specific and efficient dsRNA molecules with potentially fewer off-target effects in non-target species or within host organisms (if applied as an RNA expression construct) (Bachman et al., 2016; Taning et al., 2020).

Further, engineered synthetic miRNAs can be deployed to act as regulators in gene circuits when artificially incorporated into logic gates as internal components to sense metabolite accumulation and control flux and product yield (Quarton et al., 2018). Endogenous miRNAs can also be utilized to sense specific cellular contexts as the input of circuits because of their highly biased cell type-specific expression patterns (Liang et al., 2011; Matsuyama & Suzuki, 2020). RNA-based controllers (riboswitches)[23] have been integrated into engineered biological systems for applications spanning biosynthesis, metabolic engineering, bioremediation, health, medicine and diagnostics (Jang et al., 2018; Liang et al., 2011). Similarly, natural and engineered miRNAs have also been applied to modulate and

22 In eukaryotes, sRNA are a class of RNA molecules that include small interfering RNAs (siRNAs) derived from dsRNAs (such as from viruses), microRNA (miRNA) derived from endogenous miRNA genes (encoding hairpin structures), and other types. The size of the processed sRNAs vary depending on the species, but are roughly between 21 and 25 nucleotides in size.

23 Riboswitches are regulatory sequences of RNA with secondary structure that control gene expression through structural alterations in response to binding specific ligands without the need for sensory proteins (Bédard et al. 2020).

improve plant responses to biotic and abiotic stresses (Basso et al., 2019).

Although prokaryotes do not possess RNAi machinery, bacteria and archaea instead use sRNAs (~50 to 200 nt in size) to regulate complex networks through antisense interactions with target mRNAs *in trans*, and riboswitches, which act *in cis*, to regulate gene expression (Villa et al., 2018; Wagner & Romby, 2015). Thus, a synthetic sRNA can be designed to bind and regulate desired mRNA targets. Riboswitches are known to confer small-molecule-dependent control of gene expression, so a synthetic riboswitch can be placed downstream of a native promoter to regulate the target transgene *in cis* (Apura et al., 2019; Villa et al., 2018).

The knockout method is one of the procedures for evaluating the genetic effect on metabolite production. By deleting a gene, the effect of gene deletion on the production titre can be investigated. However, even in *Escherichia coli*, a single knockout could take weeks to create; therefore, it is not possible to study genetic effects at the genome scale. Recently, synthetic RNAs have been developed to resolve the limitation imposed by the knockout method. Essentially, they are short antisense RNAs with a considerably higher pairing efficiency than conventional antisense RNAs. Synthetic sRNAs can be used to perform a large-scale screening of genes that affect metabolite production. For improved metabolic engineering, synthetic sRNAs have recently been utilized instead of other genetic tools to downregulate genes while searching for genetic targets that could be knocked down in bacteria and improve protein production in yeast (Ren et al., 2020b; Wang et al., 2019).

Other RNA-based synthetic biology approaches rely on techniques for epigenetic modifications, such as RNA-directed DNA methylation (RdDM), which was first described by Wassenegger et al. (1994) and is like the RNAi pathway described above. RdDM is a pathway that mediates *de novo* DNA methylation, an evolutionary conserved chemical modification of cytosine bases, which exists in living organisms and utilizes miRNA. DNA methylation has been shown to be a key player in maintaining genome stability and integrity among eukaryotic organisms and contributes to the diversity in genome and developmental characteristics observed among seed plant species (Wambui Mbichi et al., 2020). A variety of tools that allow locus-specific manipulation of DNA methylation can be used to assess its direct role in specific processes, and they all rely on one of two main approaches: synthesis of siRNAs complementary to the target locus, and direct tethering of the DNA methylation machinery to the target locus through programmable DNA-binding proteins (Gallego-Bartolomé, 2020).

RNAi approaches could also be employed to support plant breeding, for instance in reverse breeding approaches (Eckerstorfer et al., 2019). Reverse breeding is a breeding technique in which plant meiotic recombination is suppressed and gametes are directly converted into adult plants (Wijnker et al., 2012). To carry out reverse breeding, meiotic genes could be engineered using RNAi, siRNAs, or virus-induced gene silencing (Dirks et al., 2009). This kind of genetic modification technique involves insertions intended to be present only at intermediate steps. Therefore, the respective modifications can be verifiably absent from the final breeding products (Eckerstorfer et al., 2019).

1.7. Modelling

Mathematical modelling plays an important role in synthetic biology because it serves as a crucial link between a concept and the theoretical realization of a particular application (Zheng & Sriram, 2010). The modelling component of synthetic biology allows the mathematical and computational design of an application and the analysis of its expected behaviour. Since modelling is based on mathematically describing a system, the biological scale of the model can also range from individual molecules to populations within an environment (Voit, 2017). For example, models that can correctly predict the behaviour of a system could allow engineers to programme new cellular behaviour without having to perform large numbers of trial-and-error experiments (Chandran et al., 2008).

In particular, the rational building of robust systems of increasing complexity from the interconnection of different parts or devices can be significantly facilitated using a forward-engineering approach relying on the separation of the *in silico* model-based design phase from the *in vivo* or *in vitro* laboratory implementation phase. This approach allows various designs to be first tested and optimized *in silico* using model-based computer simulations and mathematical analysis methods before committing any effort or time to their *in vivo* or *in vitro* realization (Baldwin et al., 2015). They enable large-scale *in silico* investigations into the robustness of specific designs, help to identify key parameters, and can filter out designs that are likely to be non-functional. This reduces the costly and time-consuming laboratory work required to develop a functional system (Pinheiro & Gorochowski, 2016). The Synthetic Biology Open Language[24] and Synthetic Biology Markup Language[25] are standards to aid the exchange of genetic design information and definition of biochemical models. Some examples of current modelling applications for synthetic biology include the design of novel enzymes (Jessop-Fabre & Sonnenschein, 2019), the computer-aided design and reconstruction of metabolic pathways (Wang et al., 2017), the automation of genetic circuit design (Nielsen et al., 2016), the prediction of microbial community dynamics (Lopatkin & Collins, 2020), modelling the confinement and reversibility of a threshold-dependent engineered gene drive (Sánchez C. et al., 2020) and the modelling of population dynamics influenced by engineered gene drives (Frieß et al., 2020), among others.

1.8. Artificial intelligence and machine learning

Many engineering endeavours involve Design-Build-Test-Learn cycles to achieve optimal solutions (figure 1). It is an iterative process with redesign based on the knowledge gained through each iteration. Using this systematic and efficient approach, synthetic biology achieves high levels of understanding of its products, becoming one of the strengths of this discipline. However, the execution of these cycles when engineering a biological system (e.g. a protein, a metabolic pathway, a genetic circuit, or a genome) has encountered multiple obstacles. One main reason is that research and development in biology is largely dependent on the artisanal work of skilful researchers (Chao et al., 2017). The Learn step has traditionally been the most weakly supported and developed, despite its critical importance to accelerate the full cycle (Radivojević et al., 2020). Machine learning[26] arises as an effective artificial intelligence (AI)[27] tool to predict biological system behaviour and empower the Learn phase, enabled by emerging high-throughput phenotyping technologies. However, it is important to note that this tool requires data sets, which are utilized to construct mathematical models (training) to identify underlying regularities or patterns, which then can provide general predictions on unseen data sets without a need to understand the detailed biological mechanisms (Radivojević et al., 2020). Machine learning has been used for example to predict pathway dynamics (Costello & Martin, 2018), DNA and RNA protein-binding sequences (Alipanahi et al., 2015), drug side effects (Shaked et al., 2016) and protein structures (Callaway, 2020), as well as for directed protein evolution/engineering (Wu et al., 2019; Yang et al., 2019).

Despite the use of AI to create accurate predictions and classifications, in most cases it lacks the ability to provide a mechanistic understanding of how inputs and outputs relate to each other (e.g. the biological mechanism that underlies a particular trait of interest). Explainable artificial intelligence is a new set of techniques that attempts to provide such

24 https://sbolstandard.org/.

25 http://sbml.org.

26 Machine learning is a branch of artificial intelligence (AI) and computer science which focuses on the use of data and algorithms to imitate the way that humans learn, gradually improving its accuracy (IBM Cloud Education, 2020a).

27 Artificial intelligence is a field that leverages computers and machines to mimic the problem-solving and decision-making capabilities of the human mind. It combines computer science and robust data sets, to enable problem-solving and seek to create systems which make predictions or classifications based on input data. It also encompasses sub-fields of machine learning and deep learning. (IBM Cloud Education, 2020b).

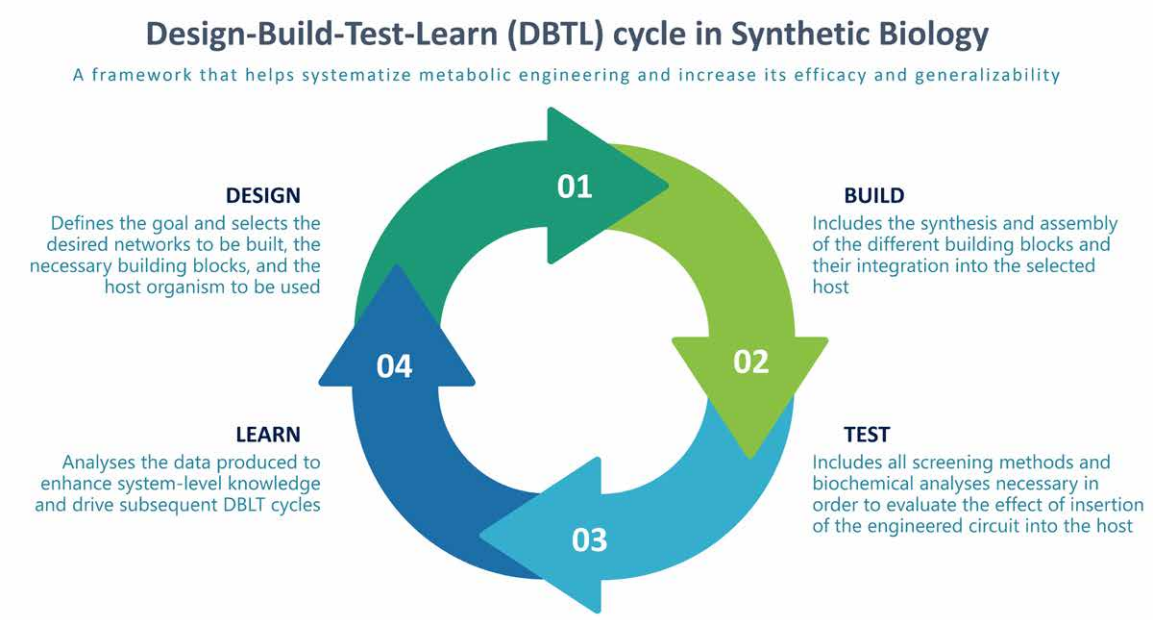

Figure 1. Iterative Design-Build-Test-Learn biological engineering cycles allow researchers to test large-scale genetic designs to enhance the process. Text adapted from Pouvreau et al. (2018).

an understanding, which is widely acknowledged as a crucial feature for the practical deployment of AI models in synthetic biology, especially as more "multi-omic" (genomic, transcriptomic, proteomic, epigenomic, metabolomic, and phenomic) data sets become available (Fellous et al., 2019; Streich et al., 2020).

1.9. Biofoundries

Automation has been proposed as a solution to improve consistency and speed in the development of synthetic parts or components, as well as to reduce labour costs and help researchers to focus more on intellectual tasks. Based on the synthetic biology enabling technologies, academic institutions and industrial companies are starting to build industrialized, integrated infrastructure (termed biofoundries) for the rapid prototyping and genetic modification of biosystems for a variety of applications. Biofoundries aim to accelerate and enhance both academic and translational research in engineering and synthetic biology by promoting and enabling the beneficial use of automation and high-throughput equipment including process scale-up, computer-aided design software, and other new workflows and tools (Hillson et al., 2019). Iterative DBTL biological engineering cycles (figure 1) allow researchers to test large-scale genetic designs and apply artificial intelligence to enhance the design process. As such, biofoundries are where synthetic biology and artificial intelligence converge to form technology platforms with the capacity to create synthetic organisms and materials on a massive scale (Dixon et al., 2020). Biofoundries provide academic laboratories and companies with cost-effective access to the high-cost equipment and small-scale prototype evaluation of others. They can significantly accelerate the engineering of biological systems by providing higher reproducibility and throughput and ease of sharing of standardized protocols (Mao et al., 2021) . Many biofoundries are being built and the Global Biofoundry Alliance, consisting of 27 non-commercial biofoundries from four continents (America, Europe, Asia and Oceania), has recently been established to coordinate activities worldwide (Hillson et al., 2019). One of these biofoundries, the London Biofoundry, has been able to quickly repurpose its infrastructure in to establish two frontline SARS-CoV-2 (*Severe acute respiratory syndrome coronavirus 2*) testing platforms, which can be quickly replicated around the world

and increase capacity for testing and drug development. According to Kitney et al. (2021), this may present a promising model for tackling future outbreaks and pandemics.

1.10. BioBricks

The foundational *Nature* paper by Endy (2005) applied three ideas from engineering to biology: standardization of basic biological parts and conditions to support their use; the decoupling of design from fabrication; and using hierarchies of abstraction so that one could work at a specific level of complexity without regard to other levels. One of the earliest and highest profile standardization systems for the design of DNA "parts" was established by scientists and engineers at MIT in 2003.[28] BioBricks™, sequences of DNA encoding a biological function, are intended to be modular parts that can be mixed and matched by researchers designing their own devices and systems.

BioBricks refers to the basic functional units that resemble one characteristic of a minimal cell and not just a particular gene or gene-product, as was the original concept from the initial synthetic biology work aimed at re-engineering host cells gene by gene. Mathematical modelling and computational tools can provide a description of such functional BioBricks to predict and guide their optimal assembly into larger functional systems (Jia et al., 2017).

A major platform for demonstrated uses of BioBricks has been the annual International Genetically Engineered Machine (iGEM) competition. The iGEM Foundation (which runs the competition) also hosts an open website, the Registry of Standard Biological Parts,[29] where researchers share the DNA sequences for parts designed following BioBrick standards. Since 2004, iGEM has provided a platform for high-school, undergraduate, and graduate students to build biological systems using existing BioBricks and designing original parts. It has since grown to include more than 350 teams from more than 40 countries competing in 2019. There are approximately 40,000 alumni of the competition, both students and instructors, worldwide, and it has been described as a source of potential commercial innovation, with more than 150 companies formed by iGEM teams (Warmbrod et al., 2020).

2. Areas of synthetic biology research

This section provides information on some of the wider areas of research. Just as with the technologies and tools supporting synthetic biology, there are also different views on what could be considered to be an area of synthetic biology research. The areas of research covered in this report and that are considered synthetic biology[30] include DNA and RNA circuits, protein engineering, metabolic pathway engineering, genome-level engineering, protocell construction, xenobiology and cell-free systems.

2.1. DNA- and RNA-based circuits

The goal of this area of research is the rational design of sequences of DNA and RNA to create biological circuits with predictable, discrete functions, which can then be combined in modular fashion in various cell hosts. Genetic circuits are seen to function as electronic logic components, like switches and oscillators (Heinemann & Panke, 2006; Lam et al., 2009).

The biological concept of predictable and programmable genetic function can be traced to 1965 with Jacob and Monod's Nobel prize-winning work on the *lac* operon in bacteria (Buc, 2016). They proposed that gene circuits with virtually any desired property could be constructed from the simple regulatory elements found in genes. Understanding the *lac* operon was key to developing a quantitative predictive understanding of gene regulation (Garcia et al., 2010; Santillán & Mackey, 2008) and laid the groundwork for future work in the engineering of

28 See https://dspace.mit.edu/handle/1721.1/21168.
29 https://igem.org/Registry.
30 Sometimes other areas of research are included within synthetic biology, such as engineered synthetic multicellularity or the design of microbial consortia that communicate across species and coordinate towards human-specified ends (Lam et al., 2009; Maharbiz, 2012). However, these areas are not discussed in the present document because they do not fall under the operational definition of synthetic biology used.

synthetic regulatory networks with predictable function. Several decades passed before the first synthetic gene circuits with predictable function were produced using simple bacterial plasmids (Elowitz & Leibler, 2000; Gardner et al., 2000).

A toggle switch or on-off switch is the simplest form of electrical circuit. A genetic toggle switch is a circuit that can produce two clearly different output states with a reversible transition between them. One of the first synthetic gene regulatory networks was a toggle switch in *E. coli* (Gardner et al., 2000). Other synthetic toggle switches have since been constructed in bacterial or mammalian cells (Atkinson et al., 2003; Kramer et al., 2004). Toggle switches in plants could be used in a variety of applications. For example, synthetic toggle switches can help regulate on-demand production of bioenergy traits such as seed oil deposition or increased biomass, or detection of pathogens or heavy metals (McCarthy & Medford, 2020). In the central dogma of molecular biology, RNA has been viewed merely as data carriers, required to translate genetic information encoded in DNA into proteins. However, the complex role of RNA in the regulation of cellular metabolism has gradually begun to be unravelled, as over the last decades numerous regulatory RNAs have been discovered. For instance, sRNA regulators modulate protein expression through base pairing, riboswitches react to the availability of certain metabolites and CRISPR serves as an immune system in bacteria. The underlying structure-function relationship makes RNA highly designable, enabling reliable construction of standardized, composable, and orthogonal parts, which can be scaled and tuned at will (Peters et al., 2015). Consequently, RNA regulators are effective tools to reprogramme existing biological systems or to build completely new ones. For instance, taking inspiration from the sophisticated circuits developed for DNA computing and self-assembly in test tubes and advances in RNA synthetic biology, Green et al. (2017) have developed RNA-only circuits in bacteria that enable complex intracellular computations to be carried out in a single circuit layer.

The design and construction of synthetic gene circuits, however, is far from straightforward — early versions of circuits rarely function as intended and typically require much post-hoc tweaking. These development efforts are hindered by a limited understanding of core design principles for gene circuits and a lack of diverse, well-characterized components for network construction. As synthetic biology extends its reach into broad application areas (e.g. health, agriculture, energy, environment) (Khalil & Collins, 2010), there is a growing need to take on these challenges to make biological design more predictable, straightforward, and time efficient. This creates opportunities for machine learning approaches (Camacho et al., 2018). For instance, a recent algorithm was developed with a limited set of training data and can predict how changes in a cell's DNA or biochemistry will affect its behaviour, then make recommendations for the next engineering cycle along with probabilistic predictions for attaining the desired goal (Radivojević et al., 2020). Among other recent developments, the construction of synthetic cell circuits can be highlighted: for instance an *E. coli* "Marionette" strain that allows the independent control of gene expression using 12 small-molecule inducers (Meyer et al., 2018), a three-cell bacterial circuit based on an ecological strategy (Liao et al., 2019), or a mammalian macrophage-fibroblast circuit (Zhou et al., 2018).

2.2. Protein engineering

Protein engineering aims to design new proteins or modify the sequence of a protein to create proteins with new or desirable functions. Strategic utilization of protein engineering methods and approaches has enabled better enzymatic properties, better stability, increased catalytic activity and most importantly, a wider range of the applicability of proteins (Sinha & Shukla, 2019). Protein engineering has a crucial role in advancing the field of synthetic biology, where metabolic engineering efforts alone are insufficient to maximize the full potential of synthetic biology (Foo et al., 2012). There are three major approaches of protein engineering research, namely, directed evolution, rational design, and *de novo* design (Sinha & Shukla, 2019). Rational design is an effective method of protein engineering when the three-dimensional structure and mechanism of the protein is well known, e.g. the computational design of transmembrane pores (C. Xu et al., 2020). In contrast, directed

evolution, a method that was awarded the Nobel Prize in Chemistry in 2018, does not require extensive information and a three-dimensional structure of the protein of interest. Instead, it involves random mutagenesis and selection to screen enzymes with desired properties, e.g. the evolution of new ribosome function by controlling orthogonal subunit interactions (Schmied et al., 2018). *De novo* design uses computational protein design algorithms to tailor synthetic proteins by using the three-dimensional structures of natural proteins and their folding rules, e.g. the ability to tune protein geometry may enable the custom design of new functions (Malay et al., 2019; Pan et al., 2020).

These methods have been used to engineer both plant-derived proteins and exogenous proteins heterologously expressed in plants (Engqvist & Rabe, 2019). For instance, plant biotechnologists are working on the evolution of *Bacillus thuringiensis* toxin to overcome insect resistance (Badran et al., 2016), the engineering of enzymes for conferring glyphosate tolerance (Mao et al., 2017; Nicolia et al., 2014; Tian et al., 2013), and the improvement of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) performance (Wilson & Whitney, 2017). Additionally, more complex protein engineering applications are starting to emerge. For example, synthetic circuits composed of interacting proteins can be designed to bypass gene regulation, interfacing directly with cellular pathways without genome modification. X. J. Gao et al. (2018) and Fink et al. (2019) engineered proteases that regulate one another, respond to diverse inputs, process signals, and conditionally activate responses in mammalian cells. These platforms should facilitate the development of "smart" therapeutic circuits for future biomedical applications (Wu et al., 2019). In another example, Bashor et al. (2019) created synthetic circuits with desired functions, based on clamp proteins with multiple protein-interaction domains, to produce non-linear behaviour from cooperativity.

To further optimize the engineering of protein, artificial intelligence is being applied. Multiple physicochemical properties must be simultaneously optimized in a broad design space of protein sequences and buffer compositions. In this context, artificial intelligence, and especially machine learning, has great potential to accelerate and improve the optimization of protein properties, increasing their activity and safety as well as decreasing their development time and manufacturing costs (Narayanan et al., 2021). These tools could unlock a further expanded range of chemistries and functions. Recent examples include a unified rational protein engineering with sequence-based deep representation learning[31] (Alley et al., 2019), a computer-aided enzyme engineering to improve enzyme-catalysed PET depolymerization (Tournier et al., 2020), and a transformed protein structure prediction using potentials from deep learning[32] (Senior et al., 2020).

2.3. Metabolic pathway engineering

Metabolic engineering seeks to optimize endemic cellular processes in specific organisms, in order to produce compounds of interest from preferably cheap and simple substrates (García-Granados et al., 2019). Synthetic biology tools make it possible to build non-natural pathways that would be difficult to produce with genetic engineering techniques. Many of the first synthetic biology commercial applications, i.e. those intended for containment, industrial processes, or laboratory settings, use metabolic pathway engineering to replicate naturally occurring molecules (Dasgupta et al., 2020). Most of the current commercially available synthetic biology products listed in section 3 fall into this category. Although initial expectations were that synthetic biology metabolic engineering would efficiently produce cheap biofuels, companies have found it easier to enter the commercial markets of higher-value and lower-volume products, such as cosmetics, active pharmaceutical ingredients, and speciality chemicals (Hayden, 2014; Keasling et al., 2012), some of

31 A machine learning method was applied to amino acid sequences to statistically represent the features of the protein. Models built upon this unified representation can be applied in protein engineering to predict the stability and quantitative function of natural, mutant or *de novo* proteins.

32 Neural networks (machine learning) were constructed to make predictions of the distances between pairs of residues, such that the structure of the protein can be more accurately predicted from a potential of mean force (free energy surface between the residues).

which are too complex to be chemically synthesized but have a value that justifies the cost of developing the relevant genetically engineered microorganism (García-Granados et al., 2019).

In pursuit of exploring various chemicals and materials as renewable resources, the metabolic engineering of microorganisms now plays an important role (Lee et al., 2012; García-Granados et al., 2019). For instance, over the course of several months, researchers created *E. coli* strains that consume CO_2 for energy instead of organic compounds. This achievement in synthetic biology, the metabolic re-wiring and lab evolution to convert *E. coli* into autotrophs, highlights the plasticity of bacterial metabolism and could enable future carbon-neutral bioproduction (Gleizer et al., 2019). Yeast metabolism has been engineered too. Researchers have achieved the biosynthesis of medicinal tropane alkaloids (Srinivasan & Smolke, 2020), the production of high-value isoprenoids in yeast peroxisomes (Dusséaux et al., 2020), the completion of cannabinoids biosynthesis and their unnatural analogues (Luo et al., 2019), and the conversion of the industrial yeast *Pichia pastoris* from a heterotroph into an autotroph capable of growth on CO_2 (Gassler et al., 2020).

In addition to microorganism hosts, plant metabolic engineering has also advanced rapidly over the last few decades. Metabolic engineering is being used to modulate endogenous metabolic pathways in plants or introduce new metabolic capabilities to increase the production of a desirable compound or reduce the accumulation of an undesirable one (Farré et al., 2014; Smirnoff, 2019). Further, metabolic engineers face greater challenges including the development of plants self-sufficient in their nitrogen requirement, enhancement of nutrients in crop plants, biofuel production from plants, plant disease control, and photosynthetic efficiency improvement (Lau et al., 2014). In the case of nitrogen fixation, Eseverri et al. (2020) have used synthetically designed genes to achieve and optimize the production of active nitrogenase protein in the chloroplasts of tobacco plants. For improving the efficiency of photosynthesis, South et al. (2019) investigated photorespiratory bypass strategies in transgenic tobacco plants to improve photosynthesis. In field trials, these transgenic tobacco plants were more productive than wild-type tobacco plants. Other advances and current challenges of engineering improved photosynthesis in the era of synthetic biology are discussed by Batista-Silva et al. (2020). Nutritional improvements are being also investigated. The Cassava Source–Sink project aims to increase cassava storage root and starch yield by metabolic engineering (Sonnewald et al., 2020). Additionally, a near-complete reconstitution of the complex biosynthetic pathway of colchicine, a plant-derived drug of historical and contemporary importance, has recently been achieved (Gleizer et al., 2019; Nett et al., 2020). The successful application of systems biology and metabolic engineering approaches in different fields of life sciences also makes it attractive for environmental scientists to use these approaches for bioremediation of environmental contaminants through microorganisms (see subsection 3.2.3(b)) (Dangi et al., 2019). Recently, machine learning is impacting the design of synthetic metabolic pathways. By predicting appropriate and significant target genes for perturbing pathway dynamics, machine learning have outperformed conventional kinetic modelling in terms of qualitative and accurate quantitative prediction, helping to optimize efforts of metabolic engineers (Choi et al., 2019).

2.4. Genome-level engineering

This area of synthetic biology research focuses on the genome as the "causal engine" of the cell (O'Malley et al., 2008; Z. Luo et al., 2018; Wang et al., 2009).[33] Rather than designing short DNA sequences or engineering specific metabolic pathways, researchers work at the whole-genome level. There are two strategies for genome-level engineering: top-down and bottom-up.

Top-down genome-engineering starts with a whole genome, from which researchers gradually remove non-essential genes to pare it down to the smallest possible genome size at which the cell can continue

33 The topic of this section and the next, on protocells, are sometimes categorized together, and sometimes top-down and bottom-up genomic engineering are separated, but all are commonly included within the scope of synthetic biology.

to function as desired, achieving a "minimal cell". The smaller genome is meant to reduce cellular complexity and thus the potential for unexpected interactions (Heinemann & Panke, 2006; Solé et al., 2007; The Royal Academy of Engineering, 2009; Glass et al., 2017; Z. Luo et al. 2018). The primary goal is to craft a simplified "chassis" to which modular DNA "parts" can be added (Lam et al., 2009). The greatest progress to date includes: JCVI-Syn3.0, a 50% gene reduction of *Mycoplasma mycoides*; several strains of *E. coli* reduced by 39% and 35% of their base pairs *in vivo*; an *E. coli* gene reduction of 78% assembled in *Saccharomyces cerevisiae*; and two 36% gene reductions of *Bacillus subtilis* (Rees-Garbutt et al., 2020).

Bottom-up genome-engineering aims to build functional genomes from fragments of synthesized DNA; it is also referred to as "synthetic genomics" (Konig et al., 2013). Thus far, researchers have reproduced the viral genomes of poliovirus (*Enterovirus C*; Cello, 2002), the 1918 Spanish influenza virus (*Influenza A virus* subtype H1N1; Basler et al., 2001; Tumpey, 2005*)*, the *Horsepox virus* (HPXV; Noyce et al., 2018), and the SARS-CoV-2 virus (Thao et al., 2020). With respect to bacteria, in 2010, the J. Craig Venter Institute published the successful synthesis and assembly of the genome of *Mycoplasma mycoides* (1.08 million base pairs long), and its transplantation into a *M. capricolum* cell stripped of its genome (Gibson et al., 2010). More recently, the completion of a 4-million-base-pair synthetic version of the *E. coli* genome was reported (Fredens et al., 2019), as well as the chemical synthesis and testing of a rewritten *Caulobacter crescentus* genome composed of the most fundamental functions of a bacterial cell (Venetz et al., 2019). Furthermore, following the synthesis of the first synthetic chromosome of *Saccharomyces cerevisiae* (Annaluru et al., 2014), the goal of the Sc2.0 initiative (Richardson et al., 2017) is to synthesize the first eukaryotic genome, the 12-Mb *S. cerevisiae* genome; this is nearing completion. Similarly, the Genome Project-Write (GP-Write) was proposed to engineer higher eukaryotes with gigabase-sized genomes (Boeke et al., 2016).

Minimization and full synthesis are just two examples of genome-scale engineering (Carr & Church, 2009). Genomes could potentially also be reprogrammed. Genome shuffling is considered as a novel whole genome improvement method (Chen et al., 2020). It was first used for strain improvement in 2002 and has been applied for phenotypic improvements of many industrially important microbial strains (Magocha et al., 2018). Other genome-level engineering methods are also emerging. Genome editing tools have enabled, for instance, the manipulation of 3D genome organization and karyotype engineering. The development of 3D genome engineering tools, such as editing of structural DNA motifs, structural proteins or manipulating DNA looping, substantially facilitates our understanding of genome organization principles and the causal relationship between 3D genome structure and functions (Wang et al., 2021). Regarding karyotype manipulation, researchers have created yeast strains with just one (Shao et al., 2018) or two chromosomes (J. Luo et al., 2018), instead of the normal sixteen chromosomes, without affecting the total number of genes, the transcriptome or growth.

2.5. Protocell construction

Similarly to the search for a minimal genome, researchers seeking to create a protocell are driven to design for less complexity at the cellular rather than the genome level. Protocells have been described as "*models of artificial cells that have some properties of living systems but are not yet fully alive*" (Armstrong et al., 2012). A synthetic protocell should be encoded by a minimal genome that specifies all essential functions and that allows the cells to thrive by coordinated transcription–translation. Such minimal systems do not contain complex networks and interactions that are present in living organisms, which creates an advantage as it allows the study of biological processes with minimal undesired interference (Exterkate & Driessen, 2019). Although the bottom-up construction of a protocell that can be considered truly "alive" is still an ambitious goal, these man-made constructs with a certain degree of "liveness" can offer effective tools to understand fundamental processes of cellular life and have paved the way for new bionic applications (Lyu et al., 2020). The construction of protocells is understood to require three things: a container or

membrane to confine reactions; a metabolism so that energy can be stored; and molecules to carry information in order to adapt to changing environments (Hürtgen et al., 2019).

Research in this area is dynamic, but thus far restricted to a basic level. Although many protocell scientists are seeking to identify new biotechnology production systems by achieving a cellular chassis, much protocell research is intended to explore the origin of life and developmental biology (Budin & Szostak, 2010; Lim et al., 2012; Exterkate & Driessen, 2019). Some recent prominent developments involve protocells with replicative fusion and division properties (Taylor et al., 2017; Xu et al., 2019), communication of artificial cellular systems (Aufinger & Simmel, 2019), shape control of vesicles (Sakuma & Imai, 2015), the discovery of the self-assembling nature of in *Xenopus* egg extracts (Cheng & Ferrell, 2019), the development of a system for transporting protein cargoes into protocells (Altenburg et al., 2020), and a scalable pipeline for creating functional novel lifeforms (Kriegman et al., 2020). Further, advances in microfluidic tools and technologies offer an engineering methodology for the bottom-up synthesis of artificial cells, by controllably generating artificial cells with precise molecular and geometrical parameters under highly controlled environments (Supramaniam et al., 2019; Ugrinic et al., 2019).

The Build-A-Cell research collaboration network[34] is facilitating studies for understanding and engineering a diverse range of synthetic cells. The Build-A-Cell network is integrating existing knowledge to engineer various aspects of biological systems, with the goal of facilitating the construction of living cells from non-living components. This network provides a formalized structure for collaboration between individual labs, bridging geographical and disciplinary divides. Other national and regional networks, such as the Building a Synthetic Cell consortium (BaSyC)[35] and the European Synthetic Cell Initiative,[36] are aimed at creating an autonomous, self-reproducing synthetic cell using a bottom-up approach.

2.6. Xenobiology

Xenobiology is the study of biological systems based on unusual biochemistries derived by chemical compounds of mostly anthropogenic origin and deliberately created in the laboratory (Pauwels et al., 2012; Budisa et al., 2020). Xenobiology aims to alter the "biochemical building blocks of life" such as by modifying genetic information to produce xenonucleic acids (XNA) or by producing novel proteins containing unnatural amino acids (Joyce, 2012; Schmidt, 2009). One approach to producing XNA is to modify the nucleotide bases of DNA beyond A, G, C, and T, incorporating alternative synthetic nucleotides into DNA (Joyce, 2012; Pinheiro & Holliger, 2012; Acevedo-Rocha & Budisa, 2016).

Candidate bases are being successfully tested for inclusion into DNA; Pinheiro et al. (2012) engineered six alternative genetic polymers capable of base pairing with DNA and polymerases that could synthesize XNA from a DNA template and reverse transcribe XNA back into DNA. Another approach to XNA is to replace the "backbone" that the bases connect to or the sugar moiety. Thus, instead of deoxyribonucleic acid (DNA), information is stored via peptide nucleic acids, glycerol nucleic acids, and flexible nucleic acids (Pinheiro & Holliger, 2012). A third approach is to modify the nucleotides' pyrophosphate leaving group (Jang et al., 2013). In 2014, a bacterium was produced where one base pair of the original DNA was altered to XNA resulting in the first organism to stably propagate an expanded genetic code (Malyshev et al., 2014). The first stable semi-synthetic organism was then reported in 2016. Researchers subsequently created a new bacterium that uses the four natural bases (A, T, C and G), which every living organism possesses, as well as a further pair of synthetic bases called X and Y in its genetic code (Zhang et al., 2017). A major milestone in xenobiology has been the creation of hachimoji DNA and RNA: systems built from eight

34 https://www.buildacell.org.
35 https://www.basyc.nl/.
36 https://www.syntheticcell.eu/.

(hachi-) nucleotide letters (-moji) that form four orthogonal pairs. This synthetic genetic biopolymer meets the structural requirements needed to support Darwinism (Hoshika et al., 2019). With double the information density of standard DNA and predictable duplex stability across all sequences, the authors suggest that hachimoji DNA has potential applications in barcoding and combinatorial tagging, retrievable information storage, and self-assembling nanostructures.

Another area of research is the production of novel proteins and proteomes that are stable but not found in nature ("never-born-proteins") (Acevedo-Rocha & Budisa, 2016). There are 20 common amino acids, but researchers have identified in the laboratory over 50 unnatural amino acids that can be incorporated into a peptide (Hartman et al., 2007). In a recent development, an unnatural amino acid (UAA) was genetically encoded at the defined site of the antibiotic resistance gene-encoded protein in *E. coli*. When UAAs were not in the culture medium, there was no expression of the antibiotic resistance gene-encoded protein. Thus, the site-specific incorporation UAA mutagenesis system could be used to control and expand the use of a conditional selectable marker, and the technique used to facilitate a rapid continuous genome editing in the bacterium (X. Xu et al., 2020). Furthermore, the first demonstration of enforcing an expanded genetic code to incorporate the rare amino acid selenocysteine to form di-selenide bonds has also been reported (Thyer et al., 2018).

Research in xenobiology is also being used to explore the basic physical properties that led DNA and RNA to be the genetic material of life (Chaput et al., 2012; Pauwels et al., 2012). Developing novel and hitherto unexplored life forms with novel features may hold great potential in almost every economic sector. A chemically modified organism (endowed with unnatural DNA bases or amino acids) that can process unnatural chemicals and convert them into something useful (a xenometabolite) would be highly desirable in the chemical industry (Acevedo-Rocha & Budisa, 2016; Nieto-Domínguez & Nikel, 2020). At the same time, XNA could provide a genetic firewall for XNA-based organisms, but not a biological firewall, meaning that XNA-based organisms may interact with DNA-based organisms on an ecological level, but never on a genetic level (Schmidt, 2010). Further, an organism that is alienated from existing ones and that relies on a certain manufactured condition to survive could be employed for biosafety purposes, thereby potentially addressing ecological concerns regarding biotechnological developments (Budisa et al., 2020).

2.7. Cell-free systems

Cell-free systems (CFS) can contribute in several ways to improving the design process of synthetic biological systems, which span scales from the molecular (genetic regulatory elements, proteins, enzymes), to the systemic (gene regulatory and metabolic networks), and all the way to extracellular levels (synthetic cells, communication, self-assembly) (Garenne & Noireaux, 2019; Laohakunakorn, 2020). First, they can accelerate DBTL cycles through rapid prototyping (Chappell et al., 2013; Niederholtmeyer et al., 2015; Takahashi et al., 2015), and second, they can be used efficiently for *in vitro* directed evolution (Contreras-Llano & Tan, 2018). They were originally conceived as tools to facilitate *in vitro* protein synthesis and consist of molecular machinery extracted from cells. They typically contain enzymes necessary for transcription and translation, and accordingly can perform the fundamental processes of the central dogma (DNA → RNA → protein) independent of a cell. The open nature of CFS means that there is no physical barrier (e.g. a cell wall) to programming and modification. CFS can be augmented with proteins or small molecules that improve the performance of synthetic gene networks (Didovyk et al., 2017; Pardee et al., 2014; Tinafar et al., 2019) or the productivity of reactions (Li et al., 2014). More importantly, genetically encoded instructions can be added directly to CFS at desired concentrations and stoichiometries using linear or circular formats. This means that conceptual designs can go from computational instructions to chemical synthesis and amplification (e.g. through PCR) to CFS without the need for selective markers or cell-based cloning steps. Such simplicity allows for rapid prototyping of molecular tools. The cell-free transcription–translation system

presents an attractive alternative to construct, characterize, and interrogate synthetic biological circuits. The cell-free transcription–translation platform known as the *E. coli* cell-extract transcription-translation (TXTL) system allows for the prototyping of synthetic circuits rapidly through iterative cycles of experiments and computational modelling (Jeong et al., 2019). TXTL has several applications, such as characterization of CRISPR elements or construction of synthetic cells. Synthetic RNA circuits are also efficiently and easily characterized in TXTL. Networks constructed from riboregulators propagate signals directly as RNAs, thus bypassing intermediate proteins, making these networks potentially simpler to design and implement than transcription factor-based layered circuits (Jeong et al., 2019).

CFS are enabling new technologies and accelerating bioengineering. In particular, some of the most active areas of research in the cell-free community are portable diagnostics, biomolecular manufacturing, and functional discovery (Tinafar et al., 2019). Cell-free synthetic biology is a promising tool to overcome inherent limitations of living cells. Its open nature enables flexible biological engineering at both molecular and cellular levels. Because cost remains a top concern in industry, cell-free biosynthesis is well suited for the development of high-value biopharmaceuticals. It is believed that cell-free systems will become more commonly used for basic and applied research in the future (Lu, 2017; Silverman et al., 2020).

3. Applications and products of synthetic biology

The advances in biotechnological tools and techniques since the late 20th century have provided diverse opportunities for use in the development of new applications and products. This section mentions specific examples to demonstrate the large and diverse range of products that are being developed, many of which are intended to alleviate biodiversity issues and to support conservation efforts (Piaggio et al., 2016). The products described below reflect prominent examples and should not be considered as an exhaustive list. It is also recognized that some of the products and applications listed in this section may not be considered synthetic biology by all readers in light of the divergent views of what is to be considered synthetic biology (see "Scope and methods" section). Further, they have been categorized by the intended environmental setting of their use:

1. *Unmanaged or wild settings* refer to uncontrolled or non-regulated "wilderness" environments. Release of synthetic biology-based applications into the wild fall under this classification.

2. *Semi-managed, managed, or urban settings* refer to partially controlled or regulated environments, and urban settlements. In these places, a combination of physical parameters and operational practices limit exposure of personnel, the immediate work environment, and the wider community to the synthetic biological application, while allowing said application to interact in the environment. The release of synthetic biology-based applications in agricultural fields, farms, zoos, or human settlements (including those rural settings where human habitation is encroaching into wilderness areas) fall under this classification.

3. *Containment, industrial processes, or laboratory settings* refer to controlled and regulated environments. In these places, a combination of physical design parameters and operational practices prevent exposure of personnel, the immediate work environment, and the wider community to the synthetic biology-based applications. The use of said applications and products in industrial or laboratory premises fall under this classification.

Within each category, products and applications are specified that are either commercially available,[37] near-term (e.g. products that have already undergone a series of regulatory approvals to advance through development, such that the next step is final regulatory

37 In this document, "commercially available" or "commercialized" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

approval for access by end-users, for example inclusion in sectorial product registers placing on the market and/or distribution to users), or in research (e.g. exploratory research, proof of concepts). For a more detailed estimation of what might be achieved in the next 20 years in synthetic biology, the "Research Roadmap" of the Engineering Biology Research Consortium (2019) can be consulted.

3.1. *Synthetic biology applications in unmanaged or wild settings*

3.1.1. *Commercially available*

No information was found of applications developed using synthetic biology approaches under this category.

3.1.2. *Near-term*[38]

No information was found of applications developed using synthetic biology approaches under this category.

3.1.3. *Research*

Applications in biodiversity conservation efforts and control of vector-borne disease

The potential for utilizing synthetic biology in conservation applications is currently being explored (Piaggio et al., 2016). There are ongoing efforts to advance engineered gene drive research to scale up efforts to protect indigenous species on islands and prevent their extinctions (e.g. Genetic Biocontrol of Invasive Rodents programme).[39] However, current research on genetic biocontrol of rodents is confined to mice due to the relative ease in manipulating the mouse genome in comparison to that of rats (Leitschuh et al., 2018). As such, the prototype engineered gene drive systems that have been recently developed for vertebrates were in mice systems (Grunwald et al., 2019; Prowse et al., 2019). Despite this, it was suggested that such systems could aid future efforts in controlling invasive species or rescuing endangered mammals (Bier, 2021).

The improvement of the resilience of wild animals and plant populations is also being explored. In ocean ecosystems, for instance, novel genetic rescue tools have been reported, especially for corals and kelp (Novak et al., 2020; Coleman & Goold, 2019). The first genome editing in coral has led to the idea of using genetic modification to increase the resilience of threatened species against anthropogenic climate change (Cleves et al., 2018). Using CRISPR-Cas9 to induce mutations, Cleves et al. (2020) have recently gained insights into the heat-tolerance of reef-building corals. Practical applications of synthetic biology to reverse or resist kelp forest loss have also been proposed, either by direct manipulation of kelp genomes or indirectly through the engineering of kelp-interacting stressor communities (Coleman & Goold, 2019). In terrestrial ecosystems, the animal model *Xenopus laevis* (the African clawed frog) has been genetically engineered with CRISPR technology to alter its immune system, a development which may enable resistance to specific amphibian pathogens (Banach et al., 2017). In another example, researchers have proposed the application of genome editing techniques to introduce plague resistance in the black-footed ferret, one of the most endangered species in the USA (Novak et al., 2018).

In addition to conservation applications, an ecological engineering project is currently under way that aims to prevent the spread of tick-borne Lyme disease by using CRISPR-based genome editing to heritably immunize white-footed mice (*Peromyscus leucopus*), the principal host responsible for infecting ticks in eastern North America (Buchthal et al., 2019). This approach is not based on engineered gene drives, but on natural inheritance of a CRISPR-based modification. However, disease prevention could also be tackled by using engineered gene drives. For example, the parasite *Schistosoma mansoni,* the causal agent of schistosomiasis, relies on snails (*Biomphalaria glabrata*) as intermediate hosts. An engineered gene drive could theoretically be designed to modify the natural snail populations

38 Engineered gene drives in mosquito for potential control of vector-borne diseases described under category semi-managed, managed or urban settings could also be considered to fit under this category depending on how and where they are used.

39 https://www.geneticbiocontrol.org/.

to confer resistance to the parasite and thus prevent its transmission (Maier et al., 2019).

3.2. *Synthetic biology applications in semi-managed, managed, or urban settings*

3.2.1. *Commercially available*[40]

(a) Genome-edited soya bean (Calyxt)

TALENs were designed to target and disrupt two fatty acid desaturase genes in soya bean. The resulting mutation causes elevated levels of oleic acid in the seeds, with no trans fats and less saturated fats, both drivers of increased risk of heart disease (Haun et al., 2014). Commercial production began in 2019 (Voigt, 2020).

(b) Biological nitrogen fertilizer for maize based on engineered bacteria (Pivot Bio)

The plant endosymbiont *Klebsiella variicola* strain 137 was metabolically remodelled using, among other tools, adaptive evolution and SDN to optimize atmospheric nitrogen fixation and resulting plant growth. Genes associated with nitrogen fixation were de-repressed (Reisinger et al., 2020; Temme et al., 2020). The product has been commercially available since 2019 (Pivot Bio Inc, 2020).

3.2.2. *Near-term*

(a) Self-limiting insects (Oxitec)

Engineered insects have been developed to contain a self-limiting genetic circuit that results in a reduction in pest insect population that either spread human disease (e.g. *Aedes aegypti*, *Anopheles albimanus*, *Anopheles stephensi*) or that damage crops (fall armyworm, soybean looper, medfly, spotted-wing *Drosophila*, diamondback moth) (Carvalho et al., 2015; Massonnet-Bruneel et al., 2013; Shelton et al., 2020).

A genetic variant of the sterile insect technique has been developed, termed RIDL (release of insects with a dominant lethal; Thomas et al., 2000), which can provide the effect of sterility without the need for irradiation, and has been developed in medfly (Gong et al., 2005), the dengue vector mosquito *Aedes aegypti* (L.) (Phuc et al., 2007), and pink bollworm (Morrison et al., 2012). These insects carry a dominant lethal gene, repressible by tetracycline (or suitable analogues, such as chlortetracycline) supplied during their larval feeding stage. The technology is designed to cause the progeny to die in the absence of the dietary additive (Alphey et al., 2010); however, sterility could be incomplete in some RIDL systems (Patil et al., 2010; Phuc et al., 2007). After release into the field, progeny die in the absence of the dietary additive. RIDL strains of fruit flies and mosquitoes were also developed in which the lethal phenotype was female-specific, termed female-specific RIDL (Labbé et al., 2012); in contrast to bi-sex RIDL, in which the lethal phenotype is expressed in both sexes (Rendón et al., 2004). This may be essential where adult females are damaging, may also provide some efficiency improvements, and may assist with managing resistance to other interventions in an integrated pest management context (Alphey et al., 2009). However, it also requires a system for sex-specific gene expression. Furthermore, the potential benefits of male-only release have not been established for moths as for various Diptera (Morrison et al., 2012).

(b) Engineered gene drives in mosquito for potential control of vector-borne diseases[41]

Engineered gene drives in disease-spreading mosquitoes are intended to spread a desired trait into a population. Usually, they harbour at least two linked sets of genetic modifications: one that confers the new trait and another that confers the ability to drive the trait into a target population (Simoni et al., 2020). Some engineered gene drive developments in mosquitoes have advanced to contained trials. By using engineered gene drive constructs, Target Malaria,[42] a vector control international research alliance, hopes to create strains of *Anopheles* mosquitoes able to

40 In this document, "commercially available" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

41 These applications could also be considered under the category of unmanaged or wild settings, depending on how and where they are used.

42 http://www.targetmalaria.org.

transmit population-suppression traits to the vast majority of its offspring, thus allowing these traits to spread quickly through the population (Barry et al., 2020). Similar initiatives, but focused on control of the parasite instead, are also under way. For instance, the University of California Irvine Malaria Initiative[43] is testing an engineered gene drive technology not to suppress *Anopheles* populations, but to prevent the transmission of *Plasmodium*, the malaria-causing pathogen in these mosquitoes (Carballar-Lejarazú & James, 2018). This approach has been tested in small laboratory cage trials of the Asian malaria vector mosquito, *Anopheles stephensi* (Pham et al., 2019).

(c) Genome-edited plants and animals

A recent review by Menz et al. (2020) estimated that 140 genome-edited cultivars of 36 crops that aim to improve yields, nutrition, resist infections and pests, and tolerate a wider range of abiotic conditions are already under development. A yield-improved maize (*Corteva*) and a better-tasting mustard (*Pairwise*) could be the first engineered products using CRISPR-Cas9 to enter the food supply (Voigt, 2020). Animals are also being subjected to CRISPR-based genome editing. Reviews by Voigt (2020), Brandt and Barrangou (2019), and Bishop and Van Eenennaam (2020) reported 67 animal examples that are being developed by genome editing, including hornless cattle, sheep with longer wool, goats that make milk with human whey protein, virus-resistant pigs, and chickens that lay allergen-free eggs. The pig genome has also been edited to allow for better sourcing of human-compatible organs for transplantation, with preclinical trials in 2020 (Voigt, 2020), which could alleviate a global shortage of transplant organs (Servick, 2019).

(d) Genetically engineered sorghum to produce a new synthetic protein to improve the digestibility in food and feed

A synthetic gene was designed such that the encoded protein, kafirin, has ten additional cleavage sites. This resulted in sorghum grain with an increased content of easily digestible proteins (G. Liu et al., 2019). A similar achievement was obtained by Li et al. (2018) using the CRISPR-Cas9 genome editing approach.

(e) Genetically engineered oilseed rape to enhance resource use efficiency of existing cropland

CRISPR-Cas9-mediated knockout lines of the genes encoding the strigolactone receptor BnD14 were transformed into rapeseed cultivar Westar. Strigolactones are responsible for the regulation of various developmental processes, including internode elongation, leaf shape, secondary stem thickening, as well as root architecture. Thus incorporation of this trait into elite breeding lines resulted in rapeseed with a tighter architecture, increased flowering and a lodging-tolerant stature amenable to responding to more inputs to improve yield (Stanic et al., 2020).

3.2.3. Research

(a) Engineered gene drive for an agricultural pest

Buchman et al. (2018) comprehensively developed and characterized an engineered gene drive system in *Drosophila suzukii,* a major worldwide crop pest. The engineered gene drive system, which has been tested in long-term, multigenerational population cage experiments, could maintain itself at high frequencies in a wild-type population.

(b) Genetically engineered bacteria for managed environmental applications, such as bioremediation, biodegradation and biomining

Bioremediation

As a potential application for phosphate removal, Liang et al. (2017) encapsulated a polyphosphate kinase in recombinant microcompartments in *E. coli*, leading to an increased uptake and compartmentalization of external phosphate pollutants. Tay et al. (2017) developed a biosensor *E. coli* strain capable of both simultaneously sensing mercury and producing mercury-absorbing, extracellular protein nanofibers. French et al. (2020) created vectors for *E. coli*, to overexpress specific hydrocarbon catabolic enzymes for the biodegradation of oil spills and, simultaneously, for horizontal gene transfer to indigenous bacterial populations for prolonged soil remediation. Additional examples, especially of clean-up of environmental

43 https://ucimi.org.

pollutants using synthetic biology approaches, are reviewed by Rylott and Bruce (2020).

Biodegradation

CRISPR has been used to enhance the activity of *Clostridium cellulolyticum* to convert cellulose into fermentable intermediates (Che & Men, 2019). This opens the possibility of constructing highly efficient cellulose-based synthetic consortia of specific fermenting bacteria to convert these intermediates into biofuels and/or bioproducts. A whole cell biodevice was recently developed for the targeted degradation of tetracycline via an engineered genetic module and optimized enzyme. Such an approach could be easily adopted and generalized for the degradation of various types of antibiotics (Xia et al., 2018).

Biomining

BioBricks-based *E. coli* strains have been developed for the adsorption of gold (Yan et al., 2018), cobalt and nickel (Duprey et al., 2014). Scientists anticipate the use of engineered microbial consortia, in part using tools of synthetic biology, to enhance mining metal recovery and to aid acid mine drainage bioremediation (Brune & Bayer, 2012). A novel method was recently announced that utilizes metal-binding peptides in fungal mycelia to enhance metal recovery from aqueous solutions such as those found in bioremediation or biomining processes (Urbina et al., 2019). Other examples of biological adsorption/chelation for biomining where synthetic biology could have an impact are reviewed by Capeness and Horsfall (2020).

(c) Genetically engineered nitrogen-fixing bacteria and other genetically engineered bacteria for agriculture

Diverse engineering strategies exist which can help design bacteria to deliver fixed nitrogen to a cereal crop (Ryu et al., 2020). The SynSym international project[44] investigates and engineers interactions between nitrogen-fixing bacteria and plants. Among many developments in this area, Yang et al. (2018) successfully transferred and expressed nitrogen fixation from *Pseudomonas stutzeri* A1501, a diazotrophic root-associated bacterium, into *E. coli*. Geddes et al. (2019) recently reported the expression of a synthetic pathway in model plants to exude bacterial signalling molecules from their roots to attract nitrogen-fixing bacteria. Another approach consists of phytomicrobiome engineering. This is an emerging field of synthetic biology to promote beneficial bacterial-plant interactions (Ke et al., 2021), as exemplified by the use of synthetic DNA to design and mass-produce custom beneficial microbes for agriculture (Waltz, 2017).

(d) Virus-induced genome editing

The USA's Defense Advanced Research Projects Agency (DARPA) Insect Allies Project is developing integrated systems of modified viral agents that can be delivered to specific plants of interest, directly to crops in fields, via insects, for combating biological and environmental threats (DARPA, 2016; Reeves et al., 2018). The first Insect Allies publication became available in 2019 and described the use of a *Foxtail mosaic virus*-based vector for somatic protein expression and somatic genome editing in model plants and maize (Mei et al., 2019). Further work using a modified nematode-transmissible virus (*Tobacco rattle virus*) encoding synthetic guide RNAs (gRNA) and *Nicotiana benthamiana* expressing the *Streptococcus pyogenes* CRISPR-associated Cas9 protein demonstrated that heritable changes to mature plant chromosomes are possible (Ellison et al., 2020).

In similar research, *Potato virus X* (PVX) and *Sonchus yellow net rhabdovirus* were modified to contain both gRNAs and Cas9 proteins. In both studies, the viral vectors were able to induce heritable changes to the host plant genome in regenerated plant tissues. Further, progeny virions recovered from infected plants were able to cause changes in newly infected plants (Ariga et al., 2020; Ma et al., 2020). More recently, an engineered PVX was used for the transient delivery of CRISPR-Cas9 components into *N. benthamiana* for efficient multiplex editing, which resulted in virus-free edited progeny (Uranga et al., 2021). In addition to inducing genomic changes, virus-induced epigenomic editing is now possible. A modified Tobacco rattle viral vector encoding gRNA was used to induce DNA

44 https://synthsym.org.

demethylation in modified *Arabidopsis thaliana* cells; however, the modified epigenetic pattern was only heritable at low levels (Ghoshal et al., 2020).

(e) Projects for the de-extinction of extinct animals
The prospect of species "de-extinction", defined as the process of creating an organism that resembles an extinct species, has moved from science fiction to plausibility within the last decade. By 2018, there were at least seven active de-extinction projects globally: the quagga (*Equus quagga quagga*), aurochs (*Bos taurus primigenius*), Floreana Island giant tortoise (*Chelonoidis elephantopus*), woolly mammoth (*Mammuthus primigenius*), passenger pigeon (*Ectopistes migratorius*), heath hen (*Tympanuchus cupido*) and an effort to restore diverse moa species (order Dinornithiformes) (Novak, 2018). Although de-extinction has not yet been achieved beyond viruses, conservationists and synthetic biologists have already begun discussing the potential impacts on biodiversity and ecosystems (Friese & Marris, 2014), and IUCN has set guiding principles on creating proxies of extinct species for conservation benefit (IUCN SSC, 2016).

(f) Transient modification of agricultural plants, pests and pathogens through RNAi spray or nanomaterials
Synthetic double-stranded RNA (dsRNA) molecules applied as a foliar spray can be designed to act as plant protection products, targeting a specific plant pest or pathogen, or introduce gene silencing within a plant. The molecules are either directly taken up by the organism or by the vascular system of the plant, which then naturally translocates the RNA molecules, to trigger a transient pest-resistant property (Cagliari et al., 2018). Transient modification of plants using topical application of dsRNA has been proven in the model *Arabidopsis* (Dubrovina et al., 2019; Kiselev et al., 2021). Various recent studies have also demonstrated that the topical application of dsRNA protects plants from aphid-mediated virus transmission, such as *Zucchini yellow mosaic virus* (Kaldis et al., 2018) or the potyvirus *Bean common mosaic virus* (Worrall et al., 2019), insects, such as Colorado potato beetle (Petek et al., 2020) or soybean aphids (Yan et al., 2020), and fungi, such as *Fusarium graminearum* (Koch et al., 2016) or *Botrytis cinerea* (Wang et al., 2016).

Synthetic DNA can also be used for transient modification of plants via nanomaterials. Grafted with DNA constructs, carbon nanotubes were used to successfully express proteins without transgene integration in *N. benthamiana*, *Eruca sativa*, *Triticum aestivum*, and *Gossypium hirsutum* leaves (Demirer et al., 2019). An efficient nanoparticle-based transient gene transformation protocol was also developed where multiple gene plasmids were expressed simultaneously in intact *Cannabis sativa* leaves (Ahmed et al., 2021). DNA nanostructures are also being assessed as a biomolecule delivery method. Zhang et al. (2019) delivered siRNA and effectively silenced a constitutively expressed gene in *N. benthamiana* leaves using such a DNA nanostructure.

(g) Genetically engineered plants to produce recombinant polyclonal antibodies against snake venom toxins
Parreño et al. (2018) described an affordable and cost-effective antivenom production based on plant-made recombinant polyclonal antibodies. This synthetic biology approach has the potential to overcome the shortage of supply of antivenoms developed from the plasma of hyperimmunized animals, the only effective treatment currently available against snakebite envenomation.

3.3. Synthetic biology applications in containment, industrial processes, or laboratory settings

3.3.1. Commercially available[45]

(a) Biopharmaceuticals

Semi-synthetic artemisinin (*Amyris*)
Yeast metabolism was engineered to express the biosynthetic pathway to produce the antimalarial compound artemisinin. Commercial production began in 2013 (Kung et al., 2018; Zeng et al., 2008).

45 In this document, "commercially available" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

Human cells for therapeutic purposes, such as for CAR-T cell therapy for cancer (*Novartis*)
T-cells are reprogrammed to express chimeric antigen receptors. The engineered proteins enable immune cells to target cancer cells (Brannetti et al., 2018; Ebersbach et al., 2020; Kochenderfer et al., 2010; Robbins et al., 2011).

Sitagliptin, a diabetes drug, using an improved transaminase from *Arthrobacter* (*Merck*)
Starting with a (R)-selective transaminase from *Arthrobacter* sp. KNK168, the protein was re-engineered using computation design and directed evolution for stereoselectivity and suitability for industrial-scale processes (Savile et al., 2010; Voigt, 2020).

Synthetic recombinant Factor C as a substitute for extracts from horseshoe crab blood (*Limulus* amoebocyte lysate [LAL]; *Lonza, bioMérieux*)
According to IUCN, the synthetic horseshoe crab blood is an example of synthetic biology application for product replacement (Redford et al., 2019). For instance, the Factor C sequence was cloned from horseshoe crabs and the protein was engineered to become active upon binding to a bacterial endotoxin. The synthetic protein can then act on a fluorescent substrate for detection similar to LAL (Bolden et al., 2020; Maloney et al., 2018). Currently, the COVID-19 pandemic has increased the need for endotoxin testing with respect to the emphasis on global vaccine production (Gorman, 2020).

Cannabinoids from engineered yeast and bacteria (*Ginkgo Bioworks, Hyasynth*)
Yeast and *E. coli* were metabolically engineered to contain the biosynthetic pathways for the synthesis of cannabinoids, cannabinoid analogues and cannabinoid precursors (Anderson et al., 2020; Bourgeois et al., 2020).

Synthetic RNA vaccines against COVID-19 (*Pfizer–BioNTech, Moderna*)
Synthetic RNA vaccines against *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) are based on nucleoside-modified RNA encoding the spike protein antigen encapsulated in a lipid nanoparticle (Chung et al., 2020; Khurana et al., 2021). A fully synthetic SARS-CoV-2 S gene was cloned in a plasmid vector, which was then used as template for the *in vitro* synthesis of the RNA vaccine (Rappuoli et al., 2021). The sequence was modified such that the translated protein would be stabilized in the prefusion conformation (Jackson et al., 2020; Vogel et al., 2021).

Viral vector vaccines against Ebola and COVID-19
Viral vector vaccines use non-pathogenic viral vectors to deliver antigen-coding DNA fragments to host cells for expression of the antigen using cellular protein-making machinery (Li et al., 2021). There are two licensed Ebola vaccines based on viral vectors: the rVSV-ZEBOV vaccine (Ervebo; *Merck*) based on *Vesicular stomatitis Indiana virus* (Regules et al., 2017), and the Ad26.ZEBOV/MVA-BN-Filo vaccine (Zabdeno/Mvabea; *Janssen)* based on Human adenovirus Ad26 and modified Vaccinia virus Ankara (Shukarev et al., 2017). For COVID-19, there are four licensed vaccines based on viral vectors:[46] one of them uses the modified Chimpanzee adenovirus ChAdOx1 (Covishield, Vaxzevria; *AstraZeneca and University of Oxford*), and the other three are based on human adenoviruses Ad5 and/or Ad26 (Ad5-nCoV, Ad26.COV2.5, and Sputnik V; *CanSinoBio, Janssen,* and *Gamaleya Research Institute*, respectively) (Mendonça et al., 2021).

(b) Carbon recycling

Processes to ferment plant waste, agricultural biogas, and industrial off-gases into petrochemical precursors (*Global Bioenergies, LanzaTech*)
A range of microbial chassis (aerobic and anaerobic, chemoautotrophic, and photoautotrophic) are metabolically engineered to convert one carbon gases into chemical precursors (Dürre, 2017; Humphreys & Minton, 2018; Karlson et al., 2021; T. W. Kim et al., 2016; Oakley, 2012; Reed & Dyson, 2013). For example, cells have been programmed to produce limonene, a jet fuel (Jansson et al., 2019).

46 WHO COVID-19 vaccine tracker and landscape: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines.

Greenhouse and waste gas (CO_2, CO, CH_4) capture and conversion into bacterial-based soil fertilizers (*Kiverdi Inc*)
Metabolically engineered bacteria capture a variety of atmospheric and waste gases to synthesize fertilizers and biostimulants for plants and fungi. The products are expected to increase soil carbon and nitrogen, as well as other nutrients (Molitor et al., 2019; Dyson et al., 2019).

(c) Fabric

Synthetic silk from microbial sources (*AMSilk, Spiber, Bolt Threads*)
Recombinant spidroins (spider silk protein) and protein fibre yarns were engineered for bacterial production, such as in *E. coli*, and enhanced qualities, such as strength, flexibility, etc. (Breslauer et al., 2018; Morita & Nakamura, 2017; Scheibel et al., 2010; Sekiyama et al., 2019; Whittall et al., 2020). In some cases, cells have also been further modified for enhanced protein production using regulatory RNAs, inducible regulatory elements, and mutagenesis (Widmaier & Breslauer, 2015).

(d) Cosmetics and fragrances

Animal-free collagen substitutes (*Geltor*)
Non-naturally occurring elastin and collagen molecules are produced by bacterial production platforms. The proteins were modified through truncation and fusion with other proteins, among other methods, to have altered properties related to melting temperature and elasticity (Ouzounov, 2019; Ouzounov et al., 2020; Persikov et al., 2020).

Synthetic squalene, an animal-free cosmetic additive (*Amyris, Inc.*)
Microbes were metabolically engineered to contain the necessary genes for the bioproduction of squalene from fermentable sugars, such as those produced by sugarcane (Fisher et al., 2009; Tsuruta et al., 2019; Gohil et al., 2019).

Nootkatone, valencene and other fragrance compounds via fermentation (*Evolva*)
Yeast cells were metabolically engineered to contain the biosynthetic pathways for nootkatone, the essence of grapefruit, and valencene, the essence from Valencia oranges (Saran & Park, 2018; Meng et al., 2020).

High-value flavours and fragrances such as vanillin, via fermentation (*Conagen*)
Microbial genes were chosen to mimic the natural vanillin biosynthesis pathway from vanilla orchids (Ni et al., 2015; Zhou et al., 2014).

Engineered yeast for producing fragrances (*Ginkgo Bioworks*)
Yeast cells were engineered to express a chimeric terpene synthase to produce terpenes (aroma compounds). The sequences for some of the engineered proteins were assembled from rare or extinct plants (Lecourt & Antoniotti, 2020; Ridley et al., 2019).

(e) Parts, devices, and systems
Synthetic biological parts, devices, and systems needed for designing genetic circuit easily are available for the synthetic biology community (from developers to amateur biologists). The Registry of Standard Biological Parts[47] and the BioMaster database have comprehensive and updated catalogues of such parts (B. Wang et al., 2021).

Expanded CRISPR-Cas systems for genome editing and diagnostics (*Mammoth Biosciences*)
Novel CRISPR proteins and systems are being discovered by applying DNA sequencing and machine learning (Burstein et al., 2017). The new genome editing systems are expected to expand our abilities to edit genomes. Further, the company is developing new diagnostic tests based on CRISPR, such as for a test for SARS-CoV-2 developed in collaboration with GlaxoSmithKline (Broughton et al., 2020; East-Seletsky et al., 2016; Mammoth Biosciences, 2018).

47 http://parts.igem.org/.

Olfactory detection devices (*Koniku*)
Konicore™ is an odour detection biosensor consisting of a microelectrode array, a microfluidics layer, and neurons that have been genetically engineered to express one or more odour receptors or other cell surface receptors. The protein receptors are in some cases additionally modified to improve and increase detection abilities (Neel et al., 2017; Renault & Agabi, 2018). To integrate and embed neurons into microfluidic devices, a nucleic acid is used to attach the modified cells to the sensor electrodes (Agabi et al., 2019). The devices have potential applications in health, security, military, and agricultural settings.

3.3.2. Near-term

(a) Engineered algae as biofactories for chemicals or renewable fuel (Synthetic Genomics, Photanol)
A systematic modular engineering of cyanobacterium *Synechocystis* sp. PCC 6803 has been recently reported to enable the highest biosynthesis of 1-butanol (a fuel substitute) production from CO_2 to date (X. Liu et al., 2019). Cyanobacteria platforms have also been used for other chemical synthesis from CO_2. For example, Diao et al. (2020) used *Synechocystis* sp. PCC 6803 to produce astaxanthin (a di-hydroxyl di-keto carotenoid) and Choi et al. (2017) modified *Synechococcus elongatus* PCC 7942 to produce squalene in a scalable photobioreactor. By applying multi-omic approaches for strain characterization and genomic manipulations (BioBricks, genome editing), algae can be metabolically engineered to contain genetic circuitry for optimized biofuel production (Benders et al., 2016; Jagadevan et al., 2018; Savakis et al., 2013; Watts et al., 2007).

(b) Biofabricated wildlife products (Pembient, Ceratotech)
Synthetic rhinoceros horns can be made to be biochemically identical to naturally sourced horn products using engineered yeast expressing recombinant keratin proteins. A DNA watermark containing a DNA sequence not naturally present in horns can be included to distinguish synthetic from natural products (Bonaci & Markus, 2019). Other synthetic horn products could be made by applying CRISPR and utilizing induced pluripotent stems reprogrammed from skin cells to mature into keratinocytes, which can then be 3D printed into a horn shape (Pandika, 2017).

(c) Cultured leather products (Modern Meadow)
Engineered yeast strains have been made which express natural or engineered collagen proteins, the molecular components of leather. The collagen sequences were sourced from animals but may be further modified for specific functional properties (Dai et al., 2019; Marga et al., 2017; Purcell et al., 2017). Cultured animal cells can also be used to produce engineered leather products (Forgacs et al., 2013).

(d) Plant-based vaccines (Medicago)
Virus-like particles (VLPs) are recombinantly produced viral structures that exhibit immuno-protective traits of native viruses but are themselves non-infectious. Synthetic biology is currently being applied to engineer VLP functions and manufacturing processes. For instance, a recent review stated that at least 97 experimental vaccines based on plant viruses have been constructed (Balke & Zeltins, 2020), including 71 vaccines against infectious agents, 16 anti-cancer vaccines and 10 therapeutic vaccines against autoimmune disorders. One example is the recombinant virus-like particles for vaccine production against COVID-19 and influenza produced in transiently modified tobacco plants. As of February 2022, one vaccine against SARS-CoV-2 (COVIFENZ®) was recently authorized for use in Canada and is likely to be commercialized soon.[48] However, vaccines against seasonal influenza have been registered, but are not yet approved in any jurisdiction (D'Aoust et al., 2016; Makarkov et al., 2019; Tusé et al., 2020).

(e) Engineered phages as antimicrobials (Eligo Biosciences)
Engineered phages have been deployed to deliver CRISPR-Cas nucleases to act as sequence-specific antimicrobials (Bikard et al., 2014; Bikard &

48 Medicago (24 February 2022) News release: Medicago and GSK announce the approval by Health Canada of COVIFENZ®, an Adjuvanted Plant-Based COVID-19 Vaccine https://medicago.com/en/press-release/covifenz/

Barrangou, 2017; Citorik et al., 2014; David Bikard & Marraffini, 2010).

(f) Engineered probiotics for the production and in vivo delivery of medicines (Precigen, Azitra, Synlogic)
Bacteria were modified to contain environmental sensing genetic circuits regulated by sensory proteins and small RNAs for the tissue-specific delivery and subsequent production of therapeutics. The modified microorganisms were also designed to excrete engineered proteins (Charbonneau et al., 2020; Claesen & Fischbach, 2015; Whitfill, 2019).

(g) Food and food ingredients

Microbial proteins for human consumption (*Motif FoodWorks, Clara Foods Co., Impossible Foods*)
Using large fermenting bioreactors, microbial cells were programmed to produce amino acids and proteins for human consumption (Matassa et al., 2016; Ivey et al., 2019; Mahadevan et al., 2020). For example, recombinant, animal-free egg white proteins can be made within modified yeast and engineered for specific food properties, glycosylation profiles and flavours (Anchel, 2020). In another example, metabolically engineered yeast *Pichia pastoris* was modified to upregulate heme biosynthesis and produce soy leghemoglobin, which improves meaty flavours and aromas when added to a plant-based burger (FDA, 2016; Fraser et al., 2018; Ivey et al., 2019; Mahadevan et al., 2020).

Bacterial protein for food and feed generated via renewable energy and direct air capture of CO_2 (AirProtein and CO_2 Aquafeed by *Kiverdi, Inc.*)
Metabolically engineered bacteria capture atmospheric CO_2 and with the assistance of H_2-oxidizing bacteria produce amino acids and proteins. Renewable energy powers the hydrolysis of water to produce H_2 gas for the hydrogen-oxidizing bacteria. The cells are then disrupted to harvest protein for use in human or animal consumption (Dyson et al., 2019; Sillman et al., 2019).

Meat from cultured cells (*Memphis Meats, Meatable, Higher Steaks*)
Cells taken from animals (e.g. cows, chickens, ducks, pigs, sheep, etc.) are cultured in a laboratory as an alternative to animal rearing and slaughter. The cells are engineered to exhibit properties for improved cell growth in laboratory settings and tissue structure for use as an edible consumer product (Genovese et al., 2015, 2018; Rischer et al., 2020).

3.3.3. Research

(a) Development of protocells and minimal cells for basic research
A protocell capable of Darwinian evolution has yet to be built, but the pieces are beginning to come together (Toparlak & Mansy, 2019; Xu et al., 2019). For instance, Huber et al. (2019) have demonstrated the feasibility of combining vesicular membrane formation and biocatalytic activity with proteins for the first time. Combining compartmentalization and biocatalytic activity enables new strategies in bottom-up synthetic biology, regenerative medicine, pharmaceutical science, and biotechnology. Further, there are international open communities that support the science and engineering of synthetic cells, such as the Build-A-Cell consortium,[49] the Building a Synthetic Cell consortium,[50] and the European Synthetic Cell Initiative.[51]

(b) Applications to produce non-native nucleotides and amino acids inside the cell for basic research
Following the announcement in 2014 of the creation of two more synthetic nucleotides (Malyshev et al., 2014), a semi-synthetic microorganism harbouring those two additional letters was reported by Zhang et al. (2017). The development of hachimoji DNA and RNA (a genetic system with eight nucleotides; Hoshika et al., 2019) has since greatly expanded the genetic code. These non-native nucleotides may have uses in barcoding and combinatorial tagging, retrievable information storage, and self-assembling nanostructures (Hoshika et al., 2019).

49 https://www.buildacell.org/.
50 https://www.basyc.nl/.
51 https://www.syntheticcell.eu/.

(c) Re-creation of extinct virus from chemically synthesized DNA fragments

Noyce et al. (2018) produced a synthetically reconstructed infectious *Horsepox virus* (HPXV) using a published genome sequence and DNA fragments manufactured entirely by chemical methods. The synthetic HPXV provided vaccine protection in a mouse model of poxvirus infection. As the use of a physical sample was not permitted by regulators, this approach to engineering a whole microbe (in this case, synthesizing HPXV *de novo*) was a way to proceed with research without compromising regulations.

(d) Genetically engineered biocontainment systems within the cell

With the rapid rise in the design of synthetic engineered organisms in recent years, the opportunity to apply them to ecosystems and human health is expected to increase. Therefore, continuous effort has been invested in designing safeguard measures to limit their dispersal (Lee et al., 2018). There are different strategies being developed to increase safety within synthetic biology. For instance, Chan et al. (2016) designed one of the first safeguard systems developed to provide programmable conditions for biocontainment, using synthetic gene systems. This synthetic gene circuit, known as the "Deadman" and "Passcode" kill switches, efficiently kills *E. coli,* and can be readily reprogrammed to change their environmental inputs, regulatory architecture and killing mechanism. The US Presidential Commission for the Study of Bioethical Issues (PCSBI) frequently mentioned "*suicide genes or other types of self-destruction triggers*" to reap the benefits of synthetic biology while avoiding potential harms (PCSBI, 2010). This is also a popular suggestion among iGEM teams to respond to biosafety concerns (Guan et al., 2013). Further, unnatural nucleotides can be used to prevent the transfer of transgenic information to wild-type organisms, as xenobiological genes cannot be used by natural organisms. This xenobiological approach would then eliminate the possibility of escape, proliferation, and cross-feeding, and render gene transfer impossible (Lee et al., 2018). Another strategy is a method known as synthetic autotrophy, where a bacterial strain is engineered to depend on a synthetic nutrient for its survival (Kunjapur et al., 2021).

(e) Digital information storage using DNA molecules

Deoxyribonucleic acid molecules have a theoretically large (petabytes per gram) storage capacity for digital data (Erlich & Zielinski, 2017). Storing data in DNA can be achieved using oligonucleotide libraries to encode the information and next-generation sequencing technologies in combination with bioinformatic pipelines to "read" the "data" (Meiser et al., 2020; Organick et al., 2018). Random access of the information can be performed using highly selective polymerase chain reactions (Organick et al., 2020; Tabatabaei Yazdi et al., 2015). Previously, a 5.27-megabit book[52] (Church et al., 2012), 739 kilobytes of computer files[53] (Goldman et al., 2013) and 35 files[54] totalling 200 megabytes have been encoded within DNA libraries (Organick et al., 2018). With the use of inorganic matrices, accelerated aging experiments have indicated that digital information encoded in DNA could potentially endure over a millennium (Grass et al., 2015). Further, the use of DNA-embedded in silica nanobeads facilitated the 3D printing of a rabbit figure containing its own manufacturing instructions (45 kilobyte digital blueprint) as well as plexiglass spectacles encoding a 1.4-megabyte video in the lenses (Koch et al., 2020). Direct digital-to-biological data storage has also been demonstrated using a CRISPR-based DNA recorder system in *E. coli* without the need to synthesize DNA *in vitro* (Yim et al., 2021).

52 53,426 words, 11 JPG images, and one JavaScript programme.

53 Five files comprised all 154 of Shakespeare's sonnets (ASCII text), a classic scientific paper (PDF format), a medium-resolution colour photograph of the European Bioinformatics Institute (JPEG 2000 format), a 26-second excerpt from Martin Luther King's 1963 "I have a dream" speech (MP3 format) and a Huffman code to convert bytes to base-3 digits (ASCII text).

54 High-definition video, images, audio, and text, including the "Universal Declaration of Human Rights" in over 100 languages (doi:10.1080/13642989808406748; http://www.ohchr.org/EN/UDHR/Pages/UDHRIndex.aspx), a high-definition music video of the band "OK Go" (https://www.youtube.com/watch?v=qybUFnY7Y8w), and a CropTrust database of the seeds stored in the Svalbard Global Seed Vault (https://seedvault.nordgen.org/).

(f) Synthetic biosensing circuits and biosensors

There is a growing need to enhance capabilities in medical and environmental diagnostics. The development of new classes of cheap, portable, and simple synthetic biology-based methods for detecting molecules of interest is proceeding, but the methods have yet to be truly adopted commercially. The range of applications being developed is broad, from environmental monitoring, toxicity assay, diagnostics, point-of-care wearable monitoring, nutrition, to food safety (Hicks et al., 2020; Slomovic et al., 2015). For sensing environmental samples, Thavarajah et al. (2020) reviewed the emerging field-deployable synthetic biology tools to sense three priority water contaminants (faecal pathogens, arsenic, and fluoride). Del Valle et al. (2021) discussed the application of new synthetic biology biosensors for use in the environment. Ravikumar et al. (2017) provided indications of how engineered microbial biosensors based on bacterial two-component systems could be used as platforms in bioremediation and biorefinery. Lin et al. (2020) reviewed portable detection biosensors with cell-free synthetic biosystems for detecting environmental pollutants. A smartphone-compatible portable biosensor that uses bacteria has been developed to detect unsafe arsenic levels (Wan et al., 2019).

3.4. Changes in synthetic biology applications and products since 2015

While considering that the applications listed in section 3 are not an exhaustive listing, a comparison between 2015 (date of publication of Technical Series No. 82 on synthetic biology) and the present document indicates that the number of synthetic biology applications commercially available and in advanced development has increased. Most of the applications continue to be related to microbial metabolic engineering and are for contained use. Certain products, such as semi-synthetic artemisinin, squalene, vanillin, shikimic acid, and select fragrances and flavours, remain commercially available. However, there is a greater availability of high-value fine chemicals, which include nootkatone, valencene and cannabinoids, among others. This marks a shift from the biofuels previously profiled. Although some biofuel products continue to be available, some companies that previously specialized in algal biofuels (e.g. Solazyme and Calysta) have since been sold or have changed their business model. Thus, the availability of these products is unclear. Developments continue with other microbes, but the focus may have shifted to bioproducing petrochemical precursors.

Further, more industries have spurred new applications not covered by the previous technical series document. For example, food proteins, textiles and materials are being produced without the need for animal sources. Similarly, wildlife products, such as rhinoceros horns, could soon be replaced with biofabricated versions. Additionally, new devices designed for olfactory detection have become available. The new tools, such as CRISPR-Cas systems, to support future developments have come to market. However, it is important to also highlight the continued availability of the Standard Registry of Parts, which plays an integral part of the iGEM competition.

Another change since 2015 is the development of commercially available products for environmental release, including genome-edited soya bean and engineered bacteria fertilizers. Other products are in advanced stages of development, such as self-limiting insects and genome-edited animals, and other have progressed from early research, such as LMOs containing engineered gene drives to control vector-borne diseases. Further, in recent years, the application of AI and the establishment of biofoundries have accelerated the research and development of synthetic biology.

However, not all progress has materialized or occurred rapidly. It is important to recognize that certain applications of synthetic biology have remained in early stages of research and development. These include de-extinction of species, biomining, plants with enhanced photosynthesis, plants engineered to fix their own nitrogen, and genetic biocontainment strategies. It is not clear when or if these developments will advance to later stages of development.

C. Potential Impacts of Components, Organisms and Products Resulting from Synthetic Biology

Parties to the Convention on Biological Diversity ("the Convention") have recognized that synthetic biology is rapidly developing and a cross-cutting issue, with potential benefits and potential adverse effects vis-à-vis the objectives of the Convention (decision 14/19). The conservation of biodiversity is one of three primary objectives of the Convention. The text of the Convention (Article 2) defines two types of conservation: (1) *ex situ* conservation, as "*the conservation of components of biological diversity outside their natural habitats*", and (2) *in situ* conservation, as "*the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties*". Notably, it is recognized that the conservation of biological diversity occurs at all levels: genes, species, and ecosystems.

Furthermore, in the context of the Convention, another of the three primary objectives, sustainable use, is defined as "*the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biodiversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations*" (Article 2). Sustainable use encompasses ecological, economic, social, cultural, and political factors (Glowka et al., 1994).

The consideration of potential impacts of synthetic biology applications on biodiversity conservation and sustainable use are therefore important aspects to be considered. Likewise, synthetic biology applications can raise social, economic, and cultural considerations of varying importance, depending upon the jurisdiction and the specific application, for decision-making and governance of their deployment. This section will therefore cover both impacts on biodiversity (section 4) and social, economic, and cultural (SEC) concerns arising from synthetic biology applications (section 5).

4. Applications of synthetic biology and their potential impacts on the conservation and sustainable use of biological diversity

Although synthetic biology is often erroneously referred to as a coherent and single discipline in more generalist texts, the developments falling under the operational definition of synthetic biology (section A) represent a wide array of potential impacts (i.e. ranging from benefits, through neutral effects and ultimately to harms) on biodiversity-related issues. In trying to describe such impacts, a multitude of factors need to be discerned. Notably, some impacts will not be unique to synthetic biology but are also applicable for any technological approach with the same aim (e.g. suppression engineered gene drives versus chemical control agents to reduce insect pest populations). Some such impacts will be specific to the host organism, while others may be related to the specific synthetic biology technique and the way that it is used. Also, some desired impacts, such as eliminating or reducing an identified population or introducing a previously extinct species, are expected to have potential secondary effects on other species with common trophic bonds, pollination requirements, host-pathogen relations etc., independent of the deployed technology. It should also be noted that although an application may be beneficial in a certain social, political, economic and/or ecological context, this should not imply that it would also be beneficial in another context (Redford et al., 2019). Potential impacts of each application should, by necessity, be considered on a case-by-case basis.

Heeding these provisos, the first part of this section discusses the potential impacts of components, organisms and products resulting from various applications of synthetic biology on the conservation and sustainable use of biological diversity. The impacts have been grouped into categories to facilitate the discussion and use of examples. Also, the discussions

have whenever possible focused on those issues raised by applications currently commercially available or approaching regulatory approval for access by end-users, as well as on applications that are widely discussed at the international level (especially the use of engineered gene drives, genome editing and RNA-based technologies in biodiversity conservation efforts and pest control). Therefore, the examples of synthetic biology applications used are not intended to provide an exhaustive coverage of the potential impacts derived from every application of synthetic biology on the conservation and sustainable use of biodiversity. Further, as previously mentioned, considering the array of divergent views held on what constitutes synthetic biology, some of the applications and examples described below may not be considered as falling under synthetic biology by all readers.

4.1. Species elimination, suppression or displacement

The idea of developing organisms containing engineered gene drives has recently emerged with potential applications not only in conservation but also in public health and agriculture (Amo et al., 2020). Specific applications are being designed to modify, suppress or eradicate populations of various target, usually pest, species (Rode et al., 2019; Scott et al., 2018), thereby increasing the feasibility of large-scale control – even with potential for continental-scale eradication – of unwanted wild populations or species (Esvelt et al., 2014; Reynolds, 2021). Currently, such applications are being developed to target invasive alien species (IAS) (e.g. invasive house mice or black rats that threaten biodiversity on islands; Leitschuh et al., 2018), human disease vectors (e.g. *Anopheles gambiae*, the main vector of malaria in Africa; Kyrou et al., 2018) and agricultural pests (e.g. *Drosophila suzukii*, a major pest of soft fruits; Buchman et al., 2018; Courtier-Orgogozo et al., 2017; Scott et al., 2018) (see section 3). Notably, many of the impacts from synthetic biology applications for species elimination, suppression or displacement discussed here remain hypothetical as none of these applications have yet been widely deployed or released. However, these developments are a response in part to the extensive toll on biodiversity (especially on islands), human welfare and crop productivity from some of the target organisms (Friedman et al., 2020), as well as some of the envisioned environmental applications potentially being beneficial to ecosystems (Kofler et al., 2018).

Arresting the adverse effects of IAS, which are a leading cause of biodiversity loss, has been a key conservation goal for decades and a priority for governments worldwide. As a consequence, there is active interest in the development of effective methods to suppress, displace or eradicate populations of IAS, especially if the new methods will produce fewer unanticipated and undesirable results than current conventional control measures (Reynolds, 2021). In addition to the management of established IAS, synthetic biology also offers novel potential approaches for the rapid response and eradication of new IAS incursions. Such approaches may be more tactical and targeted and on a smaller scale (Redford et al., 2019). In the particular case of rodent eradication, some potential benefits associated with synthetic biology approaches could include species specificity, reduced toxicant use, more humane (non-lethal) approaches and expanded application on human-inhabited islands (Campbell et al., 2015). As noted by Godwin et al. (2019), invasive rodents impact human health, food security and biodiversity worldwide; impacts on biodiversity are particularly significant on islands, which are the primary sites of vertebrate extinctions and where the limits of current control technologies are being reached. This has resulted in increased attention on potential genetic pest control approaches. However, knowledge gaps remain, for example concerning the genetic and molecular mechanisms of synthetic drive systems currently under development in mice. Furthermore, the same systems are not robustly functional in any mammal to date (Godwin et al., 2019). In addition, their use elsewhere raises additional concerns. Additional knowledge gaps relate to "translocation biology", defined by the factors influencing the survival and success of individuals newly transplanted into an established invasive population (Moro et al., 2018).

It should also be noted that the degree of impact resulting from synthetic biology applications to the management of IAS will be case-specific and vary with scale of use, context and targeted species or population (Redford et al., 2019). It is therefore possible that under certain circumstances, conservation gains from these uses could be offset or even outweighed by associated conservation losses elsewhere, for example if the target IAS performs an essential role in community structure and/or ecosystem dynamics (Redford et al., 2019). Further, depending on the type and scale of the intended and unintended modifications, LMOs containing engineered gene drives released into the environment to control an IAS, for example, could potentially also result in unwanted impacts on wider biodiversity, including off-target mutations, evolutionary resistance, ecological disturbance and extinctions, each of which have triggered discussions regarding their environmental impacts and regulatory oversight (Critical Scientists Switzerland et al., 2019; Dolezel et al., 2020; Kofler et al., 2018; Romeis et al., 2020).

These concerns over unintended consequences for ecosystems are mirrored for other applications of the technology, such as attempts to reduce populations of organisms that spread disease (Callaway, 2018). The most advanced types of application are for malaria control in which, depending on the engineered gene drive used, modified mosquitoes can pass these genes on to a high percentage of their offspring. This ensures that the modification is spread throughout the specific target populations relatively quickly and will effectively be self-sustaining (Burt & Crisanti, 2018). In addition to positive health impacts (e.g. the reduction in incidences of malaria), there could also be potential associated conservation benefits when used to complement other malaria control tools (Redford et al., 2019), for example, the reduction in the use of DDT, which was reintroduced for malaria control in 2006 under certain conditions (WHO, 2011). On the other hand, it has also been suggested that interactions with other species and gene flow (i.e. gene drive elements spreading by hybridization to closely-related species) need to be further explored on a case-by-case basis in order to assess the degree of potential negative impacts (Roberts et al., 2017). A comprehensive case study focusing on a specific population suppression engineered gene drive in *Anopheles gambiae* mosquitoes targeted for release in West Africa was recently published (Connolly et al., 2021). It identified potential harms across principal policy areas (biodiversity, water quality, human health, animal health), with the two potential harms to biodiversity comprising the reduction of densities of valued species and the reduction of ecosystem services (see section 6.1 for further elaboration of this type of risk assessment approach).

The reduction of abundance (or extinction) of the target species can have consequences, for instance for predators, competitors and prey, due to its ecological role, such as resource, consumer, competitor, or disease vector. These links create dynamic feedbacks that affect the relative abundances of different species. For example, it is estimated that around 95% of larvae of the African malaria mosquito, *A. gambiae,* are consumed before reaching adulthood (Collins et al., 2019), implying that this stage of the mosquito life cycle makes the largest contribution to the food chain. Although many predators of mosquito larvae and adults may be polyphagous, i.e. they consume a variety of prey, there are species that specialize in hunting mosquitoes (e.g. *Evarcha culicivora*, an East African spider; Wesolowska & Jackson, 2003) and as such, may be significantly impacted by the reduction in availability of *A. gambiae* (their prey). However, it has been recently noted that although this spider has a preference for blood-fed female anopheline mosquitoes, it will also feed on *Culex sp*. mosquitoes (Collins et al., 2019) and thus will only be impacted should no alternative mosquito prey populations be present. Whether many more such highly specialized predators exist remains an open question, with similar mosquito dependency by other such specialized predators requiring further investigation (Critical Scientists Switzerland et al., 2019).

In another example, it is common for populations of non-target invasive species to emerge and increase due to reduced competition or predation following control or eradication of a single (e.g. target)

species (Sofaer et al., 2018). Removing one vector/pest species could allow another potentially harmful species to take its place (niche replacement). In this respect, technologies for population replacement instead of population suppression (see below) are likely to induce less ecological harm, as the target species is still present, albeit with an extra trait, and therefore no empty niche is created (Kopf et al., 2017; Rüdelsheim & Smets, 2018; EFSA GMO Panel, 2020). Moreover, the suppression of the invasive alien or pest population may harm non-target organisms that rely on the target species for the delivery of ecosystem services (such as pollination, decomposition, etc.) (Romeis et al., 2020). Further, although not specific to synthetic biology approaches, the reduction or elimination of human malaria from geographical areas may lead to demographic and land-use changes, potentially impacting biodiversity conservation (Redford et al., 2019). Thus, each of these potential impacts may/should be considered during regulatory decision-making (see subsection 5.1.1).

In attempting to understand the extent of any impact on biodiversity, it is therefore important to differentiate between the types of engineered gene drive system being utilized, as the application could theoretically be designed to either spread through target populations (non-localized) and persist indefinitely (self-sustaining) or be restricted in spread (localized) or persistence (self-limiting) (Devos et al., 2020; Harvey-Samuel et al., 2017). Further, such applications with engineered gene drives may be used in two ways, depending upon the effector gene contained within the drive construct (see Devos et al., 2020; EFSA GMO Panel, 2020; Rüdelsheim & Smets, 2018):

- Those containing a suppression drive: Used to eliminate invasive species, suppress populations of human and animal disease vectors, and to control agricultural invertebrate pests such as fruit flies, moth pests, thrips and mites;

- Those containing a replacement drive (also termed modification drive): Used to provide an extra trait to the target population, e.g. in endangered species or crop and livestock breeding, or to block pathogen development.

A distinction should thus be made between those applications attempting to suppress a population and those attempting to replace a specific trait within a population, as they have different implications for potential environmental interactions. Further, the survival in the wild of an LMO containing an engineered gene drive will depend upon, among other things, how well it can compete with its wild relatives and its susceptibility to mutations that either lead to the loss of the desired trait or that disrupt the introduced change. Limiting the scale of exposure of the target organism in the environment, either by the number, timing or location of releases, may minimize the extent of impact (Brandenberg et al., 2011).

Recent research has indicated that engineered gene drives may face resistance and thus limited efficacy in wild mosquito populations. Resistance towards engineered gene drives is an important concern, especially for homing endonuclease and RNA-based methods. These are sensitive to the evolution of resistance alleles via cleavage repair by non-homologous end joining (NHEJ), *de novo* mutations, or genetic variability (e.g. polymorphisms) in their recognition sites that become immune to conversion by the drive system and thus likely to affect the spread of drives based on such mechanisms (Hammond et al., 2017; Rode et al., 2019). It is almost inevitable that resistance should evolve against standard engineered gene drives approaches in most natural populations, unless NHEJ can be effectively suppressed, the fitness costs of the driver are completely dominant, or these fitness costs are on par with those of resistance alleles (Unckless et al., 2017). Thus, in the case of suppression drives, it is expected that there will be greater evolutionary pressure. However, it is uncertain how rapidly modification drives will spread into the wild owing to lessened selective/evolutionary pressure (Critical Scientists Switzerland et al., 2019).

Highly invasive self-sustaining "global" drives may be problematical whenever a drive is required to be confined to a specific population, such as an island or a continent (Esvelt & Gemmell, 2017). By contrast, self-limiting drives such as TARE (toxin-antidote recessive embryo) with an invasion threshold could remain confined to contiguous populations without

being able to invade sufficiently distant populations through occasional migrants. By this means, they may also provide a critical component in enabling so-called "tethered" drives, which could be used for both population modification and suppression strategies (Champer et al., 2020). So, the concern that engineered gene drives, once released, would potentially act globally should therefore be nuanced and will be case-specific; they may very well spread through different populations but not necessarily achieve very high frequencies in each (Rüdelsheim & Smets, 2018). Further, Gomulkiewicz et al. (2021) have recently used *in silico* modelling to identify molecular strategies that may potentially avoid the evolution of resistance to engineered gene drives and allow eventual population suppression to expected levels.

Depending on the intent or perspective of each application containing an engineered gene drive, persistence in the environment could be considered either a positive or negative feature. Self-limiting gene drive-engineered organisms might persist for a maximum of only a few generations whereas other self-sustaining gene drive-engineered organisms may persist for years. Geographic spread is of course related to persistence. An engineered gene-drive organism that has been designed to spread will not get very far unless it can persist in the wild (EFSA GMO Panel, 2020; Friedman et al., 2020). There have, however, been concerns raised over the lack of controllability in that certain gene drives are likely to be "highly invasive" and to spread to most interbreeding populations (Noble et al., 2018; Sirinathsinghji, 2019). It has also been noted that if non-localized drives do propagate as intended, although spread would be limited to the distribution of the target species, this could be a large geographical area indeed and therefore there is uncertainty about the numbers of populations that might be affected (Critical Scientists Switzerland et al., 2019). Given that certain LMOs containing engineered gene drives can potentially impact biodiversity, national sovereignty and food security, there is a need to develop strategies to minimize any potential risk, including those of intentional and unintentional spread and to mitigate any potential harm to humans or the environment (de Wit, 2019; DiCarlo et al., 2015; National Academies of Sciences, Engineering, and Medicine, 2016b).

4.2. Improved agricultural performance

One area receiving intensive attention from developers using synthetic biology techniques centres around the use of resources in agriculture, from strategies to reduce/replace chemical inputs (e.g. Pivot Bio's biological nitrogen fertilizer, Oxitec's genetic biocontrol insects), to those that increase nitrogen fixation efficiency in crop symbiotic bacteria (Ryu et al., 2020) or that improve yields of current cropping areas through modified crop architecture (Stanic et al., 2020). Additional potential benefits are also case-specific and may include enhancement of decomposition rates and nutrient fixation; reduction in the application of fertilizer; more efficient production of farm animals with concomitant reductions in feed and land use; forest restoration; and production of livestock feed based on more efficient industrial production of microbial proteins (Redford et al., 2019). Notably, some have clear similarities to impacts already considered from analogous applications exploiting genetic modification technology in agriculture. Although it has been reported that there is little evidence to connect the latter with adverse agronomic or environmental problems (National Academies of Sciences, Engineering, and Medicine, 2016a), potential impacts from certain applications may include effects from transferring genetic material to wild populations; having toxic effects on other organisms; creating new invasive species; facilitating greater application of agrochemicals with consequent biodiversity impacts; and reducing soil fertility and structure by allowing more intensive agriculture (Science for Environmental Policy, 2016).

Some of the techniques of genome editing (which for some readers may not fall under synthetic biology; see "Scope and methods" section) are less precise than others, such that molecular changes additional to those intended (i.e. off-target modifications) can also be introduced into the host organism; again, phenomena that have already been reported with genetic engineering (Eckerstorfer et al., 2019) as well as conventional breeding. In general, several types of these off-target modifications in genome-edited

plants can be distinguished (Agapito-Tenfen et al., 2018):

- Changes at genomic locations other than the intended genomic target site(s), i.e. modifications which are usually not genetically linked to the desired trait(s);

- Molecular changes in the vicinity of the intended site of modification, i.e. changes different from the intended modifications, but tightly linked to the desired trait(s);

- Effects different to the desired trait(s) which are due to the modifications at the genomic target, i.e. pleiotropic effects of the intended modification(s) linked to the desired trait(s).

To evaluate the consequences of such off-target effects, it is necessary to compare genome editing with conventional plant breeding. For example, recent experimental evidence indicates that off-target mutations potentially induced by NHEJ-based, indel-producing, CRISPR-Cas genome editing techniques are very unlikely to occur at genomic sites without sequence homology to the target site in plants (Singer et al., 2021), and those that do occur are of the same type as those mutations obtained through conventional breeding (EFSA GMO Panel et al., 2020). Further, the mutagenesis techniques[55] used in conventional breeding are rife with off-target effects that few people ever bother to detect or characterize. These previously have not been a cause for safety concerns, and there has been a history of safe use of mutagenized crops (Duensing et al., 2018). Thus, while it is possible to optimize the editing process to minimize off-target effects, many off-target modifications can be identified during the breeding/product development process and if unwanted, can be counter-selected or targeted for removal so that they are not present in the final product. Those off-target changes that remain may or may not lead to phenotypic effects affecting the properties of the modified organism (European Commission High Level Group of Scientific Advisors, 2017), and those that do may have the potential to ultimately lead to alterations of population characteristics, especially when spread among individuals via gene transfer. This may ultimately lead to unintended or unexpected consequences during interactions with associated species or populations in the surrounding environment and which would be evaluated during any risk assessment (see section 6). Conversely, the cause of some of this imprecision could also be exploited by developers to intentionally modify more than one related sequence (with less than 100 percent sequence identity) in attempts to modify different alleles or homologous genes in the host organism at the same time (Lema, 2021).

Concerns have also been raised surrounding the generation of plant allergens, toxins and anti-nutrients, which may pose a risk to human and animal health (African Centre for Biodiversity, 2020; Zhao & Wolt, 2017); however, the latter publication also indicates that the technology *per se* does not increase the likelihood of causing such a deleterious characteristic. Additional concerns are that unintended on-target and even precise edits could potentially result in DNA misreading, which in turn could affect protein composition and thus safety of the plant in the environment (African Centre for Biodiversity, 2020; Canadian Biotechnology Action Network, 2020), again, an aspect that would typically be evaluated during case-specific risk assessments (see section 6.1.).

4.3. Climate change challenges and environmental solutions

As is the case for other environmental challenges, synthetic biology has the potential to help tackle challenges from climate change. In particular, the planet is experiencing major disturbances in important ecosystems, including forests, fire-prone regions and coral ecosystems. Food production is also being threatened by extreme weather events that were once rare. These environmental changes are even more significant considering that the long-term temperature consequences will remain substantial even if CO_2 emissions stop immediately, as the

55 Chemical (e.g. alkylating agents, intercalating agents, base analogues) and physical (e.g. gamma rays, X-rays, ionizing radiation) mutagenesis are considered techniques used in traditional plant breeding (Oladosu et al., 2016).

timescale for temperature reduction by natural processes is in the order of a thousand years (DeLisi et al., 2020). Synthetic biology offers the possibility to help address some of these challenges on a decadal timescale (DeLisi, 2019).

An example of a current environmental challenge associated with climate change and anthropogenic stressors is the global decline of coral reefs. With coral bleaching events predicted to be more frequent and severe, adaptation to warming oceans will therefore be critical to maintaining ecosystems under new environmental conditions (Anthony et al., 2020; Matz et al., 2018). As described earlier (subsection 3.1.3), researchers have applied CRISPR-Cas genome editing to reef-building corals, leading to an increased understanding of thermal tolerance (Cleves et al., 2018, 2020). Such advancements in "facilitated adaptation" could lead to reef restoration programmes in the future (Reynolds, 2021), for example, by modifying corals in Australia's Great Barrier Reef to better withstand warmer and more acidic marine water, abiotic aquatic characteristics which result from elevated atmospheric concentrations of greenhouse gases. While considerable technological development is still required before these methods can be applied to corals and their microbial symbionts, these early achievements suggest that they may be available in the future (Redford et al., 2019).

It has been suggested that climate adaptation in species that serve key ecosystem functions could protect some of the services that reefs provide, such as providing the infrastructure for over 1 million aquatic species (Anthony et al., 2017). Moreover, if the species chosen are functionally redundant, the potential positive impacts of engineered reef restoration can be maximized (Oppen et al., 2017). On the other hand, since it is not feasible to engineer millions of species, prioritizing specific species for interventions would not protect the underlying integrity and diversity of coral reef ecosystems, which could have implications on the services provided (Anthony et al., 2017). Also, due to the nature of the introduced traits, there could be concerns that the engineered corals will have a competitive advantage within the environment, consequentially leading to an overall decrease in genetic diversity (Filbee-Dexter & Smajdor, 2019). Further, with the application of genome editing techniques in marine resources, there are concerns that off-target genomic changes could lead to unwanted effects within the organism and ecosystem, something that remains to be examined as no applications have been deployed to date (Redford et al., 2019; Blasiak et al., 2020; Spalding & Brown, 2015). Similarly, concerns have been raised regarding potential effects on non-target populations, which could arise should genome-edited stages disperse from the populations targeted for management to other populations of the same coral host or symbiont (Redford et al., 2019).

Concerning carbon emissions, climate change due to these emissions has been linked to losses in biodiversity (SCBD, 2020b). Thus, there is a greater need to reduce greenhouse gas emissions in many areas, including the industrial production of chemicals and fuel. Potential impacts are linked to claims that there could be significant benefits for biodiversity from replacing fossil fuel energy sources with bioenergy, based on the premise that these approaches could reduce global dependency on fossil fuels and cut harmful emissions at a significant scale (PCSBI, 2010). In this sense, synthetic biology is being used in designing "next-generation" biofuels (subsections 3.3.1(b) and 3.3.2(a)) that, it is hoped, will overcome challenges of "first generation" biofuels made from food crops (Jeswani et al., 2020; Royal Academy of Engineering, 2017); they could then replace petrochemical sources or mitigate emissions caused by their combustion (Köpke & Simpson, 2020).

Potential negative impacts could result from the increased utilization of biomass for synthetic biology applications. "Biomass" is generally used to refer to the use of "*non-fossilised biological and waste materials as a feedstock*" (ETC Group, 2011; Jeswani et al., 2020). Additionally, potential negative impacts include the displacement of sustainable uses of biomass, the destruction of native forests and marginal lands such as deserts and wetlands to provide land to establish plantations for biomass production, and harvesting of biomass from natural grasslands

(ETC Group, 2010; Royal Academy of Engineering, 2017). On balance, many anticipate that the potential efficiencies and attendant reduction in reliance on fossil fuels offered by energy production using synthetic biology would offset anticipated risks to the environmental ecosystem as it exists today. But uncertainty remains (ETC Group, 2015).

One example could be through the use of engineered algae, which could use light and atmospheric carbon dioxide to produce biofuels (e.g. Photanol). There are two main methods of growing algae: open ponds and photobioreactors. If open ponds are used and there is the escape of engineered algae into the environment (Shuba & Kifle, 2018), there is the potential for nutrient depletion and a resultant reduction in local biodiversity; increased fitness advantages to out-compete native species; and/or the production of toxins linked to algal blooms (Abdullah et al., 2019). Under contained conditions, such as production in photobioreactors, it would be likely that potential direct negative environmental impacts would be minimized as production conditions would be more strictly controlled (Shuba & Kifle, 2018). Once again, potential impacts and the need for any necessary management procedures would be assessed prior to any authorized use (subsection 6.1.1). It is also important to consider that these operations could be energy intensive and produce higher life-cycle emissions (Jeswani et al., 2020).

Beyond algal platforms, microbes can also be engineered to "recycle" greenhouse gas and industrial off-gas emissions to create chemical precursors and biofuels. For example, it was estimated that using a LanzaTech fermentation process, where microbes fermented gases to produce ethanol, greenhouse gas emissions could be 60 and 90% lower than from conventional fossil gasoline, depending on whether the source was industrial off-gas or fermented biomass (switchgrass, maize stover, forest residue) (Handler et al., 2016). Emission reductions can also be observed for petrochemical precursors when microbes incorporate atmospheric greenhouse or waste gases.

Moreover, applications for food and feed protein production (subsection 3.3.2(g)) could have potential positive impacts on water and land use. For example, the production of protein by microbes using renewable energy sources would use ten times less water and land, as compared to typical soya production in the USA (Sillman et al., 2019). Additionally, this would result in a concomitant removal of the need for both particular chemical products (fertilizers, pesticides and herbicides) and arable land. Land use dependency could also be further minimized depending on the type of renewable energy source used (i.e. wind vs. solar power) (Sillman et al., 2019).

4.4. Replacement of natural materials

Unsustainable international commercial trade in wildlife, whether legal or illegal, is one of the greatest threats to wildlife today. One approach to supplying markets while taking pressure off wild populations is to provide substitutes for wild-caught species (Redford et al., 2019). Examples include recent CITES permits issued in China to allow two synthetic biology projects to investigate the use of microbial cell cultures to produce the plant-derived compounds taxol and ginseng (CITES, 2018). Additional examples from section 3 include the production of recombinant Factor C (rFC) from synthetic horseshoe crab blood, and synthetic rhinoceros horns and squalene, each of which could reduce or remove the need to exploit wild species (ETC Group, 2013; Woodrow Wilson International Center for Scholars, 2012). Should the synthesized item be a suitable substitute for the wild product, this could be positive for conservation, taking pressure off the wild species and their natural habitats while supplying market demand.

However, it was recently flagged within the CITES community that there may be a need to consider creating rules for specimens produced from synthetic or cultured DNA as the demand for them could not only lead to an increase in the demand for (illegal) natural specimens (e.g. rhino horn, ivory, pangolin scales, medicinal plants, fragrances, etc.) but they could also be mixed with (illegal) natural specimens. It could be detrimental to the aims of CITES to protect species in the wild if synthetic alternative specimens fall out of the scope of CITES (CITES, 2018).

One case where real-life experience has been gained concerns vanillin. Initially, the production of vanillin by synthetic biology (subsection 3.3.1(d)) raised concerns that its large-scale production could negatively impact the many smallholder farmers involved in the production of cured vanilla beans (ETC Group, 2013). Vanilla orchids are commonly produced by intercropping with rainforest trees as "tutors" for vanilla vines to grow on, and so it was thought that reduced demand for the natural product could disrupt this agro-ecological method of cultivation (ETC Group, 2013). The developers of vanillin, on the other hand, claimed that their product offered the world a clear alternative to the petrochemical variety of vanillin without introducing a new environmental threat to rainforests and endangered species. A recent report by United Nations Conference on Trade and Development (2019) supports the latter claim. It appears that naturally sourced vanilla remains highly valued, as consumers prefer its more complex flavour profile. As a consequence, UNCTAD expect that the naturally sourced product will continue to have appeal, and therefore vanillin may not have a significant impact on the *in situ* conservation of the natural product. Please note that economic concerns are addressed later, in subsection 5.2.2.

5. Social, economic and cultural concerns from applications of synthetic biology

The use of synthetic biology triggers a wide variety of views related to perceptions of risks and benefits, moral and ethical values, along with broader issues such as socioeconomic aspects. A science-based assessment of impacts is therefore usually seen as part of a wider decision-making activity; one that may include the evaluation of such economic, political, moral and ethical concerns alongside scientific predictions of changes that would result from using technology. Voluntary guidance on the process for assessing such concerns in the context of reaching a decision on LMO import per Article 26 of the Cartagena Protocol on Biosafety is available (SCBD, 2018), especially with regard to the value of biological diversity to indigenous peoples and local communities. The document also provides an operational definition and lists important principles for the process of assessing socioeconomic effects. Concerns from those effects that have gained greater credence with the emergence of synthetic biology applications reaching the later stages of development are elaborated further in this section.

5.1. Societal concerns

5.1.1. Incorporating societal concerns into regulatory decision-making

As new applications hold promise to address global challenges related to the environment, conservation, climate change, health and more, international discussions concerning synthetic biology have come to the forefront, are now much more visible and have drawn attention from a wide range of actors. Their interests and concerns which extend beyond those addressed during a risk assessment, also have a role to play in regulatory decision-making concerning activities involving synthetic biology. Typically, such decisions would be taken by risk managers or decision makers, given the characterized risks and potential prescribed risk management strategies. However, the degree to which a risk is acceptable cannot be determined purely scientifically; science can predict the likelihood of certain effects, but non-scientific criteria must be included in the process of judging their acceptability (Johnson et al., 2007). Thus, the acceptability of any risk is a social construct, as are the guiding policy goals, and should be informed through consultation with a broad set of stakeholders (Craig et al., 2017; Devos et al., 2019, 2020), including the populations likely to be impacted most (subsection 5.1.2). When it comes to the use of the technologies and their applications covered in this document, national, regional, and international governmental agencies are working to clarify how existing research policy, field testing frameworks, and risk assessment guidelines may apply to their environmental uses, by enacting some existing rules, and seeking to update and create new policies to address these technologies. Largely missing from these activities, however, is attention given to local communities in regulatory decision-making, even though they are the most likely to feel a

potential impact from these applications (Kofler et al., 2018). Conversely, to be best prepared to address any concerns from society, the synthetic biology community should be aware of, and respond to, these challenges by engaging in horizon scanning exercises as well as open dialogue with regulatory bodies, the media and the public (El Karoui et al., 2019). Echoing this sentiment, the OECD has recently published a draft OECD Recommendation on Agile Regulatory Governance to Harness Innovation[56] for public consultation, among which it is highlighted "*that Adherents put in place mechanisms for broad public and stakeholder engagement in the regulatory process, including citizens and innovative small and medium-sized enterprises (SMEs), from an early stage and throughout the policy cycle to enhance transparency, build trust and capitalise on various sources of expertise*".

It has been suggested that for applications of emerging technologies that affect the global commons,[57] concepts and applications should be published in advance of construction, testing, and release (Oye et al., 2014). They argue that this lead time would enable public discussion of environmental and security concerns, research into areas of uncertainty, and development and testing of safety features. It would also allow the adaptation of regulations in light of emerging information on benefits, risks and policy gaps, and, more importantly, it would allow broadly inclusive and well-informed public discussion to determine if, when, and how some applications (e.g. engineered gene drives) should be used (SCBD, 2015; SCBD, 2018). In a similar vein, the public, in the form of consumers, were also identified in a recent survey of experts by Lassoued et al. (2019), as playing a major role in determining where and how emerging technologies, in this case new breeding techniques[58] which include genome editing techniques, will be developed and used in agriculture (Obukosia et al., 2020; Seyran & Craig, 2018).

Recent activities in public engagement on the topic included a survey of the public's views in the USA in which 15% of respondents considered themselves informed about synthetic biology while only a slightly higher percentage (20%) that synthetic biology was of personal importance to them, suggesting that the individuals did not perceive the research area as having much personal relevance (Akin et al., 2017). The same report indicated that "*education, religiosity, deference, scientific knowledge, risk–benefit perceptions, and trust in scientists all correlate with individuals' attitudes towards synthetic biology*". In another recent national survey, CSIRO's Synthetic Biology Future Science Platform began measuring the Australian public's attitudes towards synthetic biology (CSIRO, 2021). It showed that 85% of respondents had little or no knowledge of synthetic biology and its applications, with the majority expressing interest in knowing more. Most preferred passive information exchanges (e.g. receiving results and feedback through social media) for learning more, with a specific focus on the possible risks and how they would be managed. The same survey identified moderate to high overall support of the example synthetic biology technologies (e.g. for restoring the Great Barrier Reef, managing invasive pests, changing the properties of natural fibres, etc.); however, support was highest when there was a public health need (e.g. managing mosquito-borne viruses) or an environmental benefit (e.g. protecting Australia's biodiversity). The analyses indicated that "*public support may be driven by: emotion; perceived benefits; advantages of the technology compared to current solutions; efficacy of the technology; and trust in science*" (see subsection 5.1.2 for more on community engagement).

Society as a whole therefore has a key role to play in helping decision makers and regulators better define specific protection goals (or "assessment endpoints"), i.e. the things that society doesn't want harmed

56 A public consultation on the draft OECD Recommendation on Agile Regulatory Governance to Harness Innovation closed on 2 July 2021 (https://www.oecd.org/gov/regulatory-policy/public-consultation-on-the-draft-recommendation-for-agile-regulatory-governance-to-harness-innovation.htm).

57 One definition of the global commons currently used by international law names the high seas, the atmosphere, Antarctica, and outer space as the globally common resources that fall outside national jurisdictions (Nakicenovic et al., 2016).

58 New breeding techniques include genome editing (CRISPR, ODN, ZFN, TALENs), cisgenesis, intragenesis, RdDM, agroinfiltration, grafting LM rootstock and reverse breeding, among others (Seyran & Craig, 2018).

(section 6); that then dictates the characteristics of new products or technologies from synthetic biology to be assessed scientifically (Craig et al., 2017) and the potential impacts to also be assessed socio-economically (SCBD, 2018). Then, once the degree of potential harm from the specific case has been assessed, society can once more be key to guiding decision makers and regulators *a priori* in explicitly determining the extent to which that harm may be acceptable (or not) before any authorization is considered (Office of the Gene Technology Regulator, 2013). This would be extremely helpful, especially in cases where policy objectives are in conflict or where policy trade-offs may be necessary, e.g. the suppression of disease vector populations (human health versus biodiversity/environmental protection), the cultivation of improved crop varieties in centres of origin (food security versus biodiversity/environmental protection), product substitution through the replacement of naturally-harvested products with those resulting from synthetic biology (eco-friendly production/environmental protection versus livelihoods of smallholder farmers/rights of indigenous peoples), etc.

5.1.2. Indigenous peoples and local communities (IPLCs) and community engagement

Mirroring the above desire for more explicit societal involvement in regulatory decision-making, the concept of free, prior and informed consent (FPIC) has grown steadily in prominence in the context of conservation and land development decisions impacting IPLCs. Originating from international human rights standards associated with the right to self-determination,[59] it has evolved in the context of decisions that threatened the removal of indigenous peoples' communities from their lands and territories and has been explicitly adopted in certain international instruments that recognize the plights of indigenous peoples and defend their rights (George et al., 2019), initially by the Indigenous and Tribal Populations Convention, 1957 (No. 107)[60] of the International Labour Organization adopted in 1957 as revised in 1989 by the Indigenous and Tribal Peoples Convention, 1989 (No. 169),[61] and subsequently by the Convention on Biological Diversity in 1992 and the United Nations Declaration on the Rights of Indigenous Peoples adopted by the United Nations Assembly in September 2007 (see subsections 9.3.2(a), 8.1 and 9.3.2(b), respectively).

These instruments reflect FPIC as an evolving concept and have supported an upsurge in initiatives focusing on participatory development and indigenous inclusion by international and national development-focused agencies and organizations. For example, the *Free Prior and Informed Consent* manual from FAO was designed to enable field practitioners to incorporate FPIC into the design and implementation of projects and programmes, ensuring that the rights of indigenous peoples are duly respected. Further, the manual notes that elements within FPIC are interlinked and should not be treated as separate elements (FAO, 2016). Similarly, a 10- to 12-year process under Article 8(j) of the Convention on Biological Diversity (see subsection 8.1.7) resulted in the Mo'otz Kuxtal Voluntary Guidelines for the development of mechanisms, legislation, administrative or policy measures or other appropriate initiatives to ensure "prior and informed consent", "free, prior and informed consent" or "approval and involvement" of IPLCs, for a fair and equitable sharing of benefits arising from the use and application of traditional knowledge (SCBD, 2019a). Simply stated, consent or approval should be sought before any project, plan or action takes place (prior); should be independently decided upon (without pressure or manipulation; free); and should be based on accurate, timely and sufficient information provided in a culturally appropriate way (informed) for it to be considered a valid result or outcome of a collective decision-making process (FAO, 2016; SCBD, 2019a). Further, FPIC is not just a result of a process to obtain consent to a particular project; it is also a process in itself, and one by which

59 The right to self-determination is a fundamental principle in international law, embodied in the Charter of the United Nations and the International Covenant on Civil and Political Rights and the International Covenant on Economic, Social and Cultural Rights.

60 https://www.ilo.org/dyn/normlex/en/f?p=NORMLEXPUB:12100:0::NO::P12100_ILO_CODE:C107.

61 https://www.ilo.org/dyn/normlex/en/f?p=NORMLEXPUB:12100:0::NO::P12100_ILO_CODE:C169.

IPLCs are involved through their full and effective participation in discussions and decision-making (FAO, 2016; SCBD, 2019a). Additionally, "prior informed consent" was also enshrined in the Akwé: Kon Voluntary Guidelines, which were developed under the Convention for the conduct of cultural, environmental and social impact assessments related to developments that would take place on or likely impact sacred sites, and on lands and waters traditionally occupied or used by indigenous and local communities (SCBD, 2004).

Despite the increasing awareness of, and resources available to support, participatory decision-making processes, translating FPIC into practice across national, state, or provincial contexts of land and resource governance has proved challenging (George et al., 2019), and the Convention on Biological Diversity is no exception. Enshrined in its preamble and provisions is the recognition of the dependence of IPLCs on biological diversity and their unique role in conserving life on Earth. Further, issues concerning IPLCs are reflected in the recommendations and decisions of bodies at all levels of the Convention, for example, the 2017 AHTEG on Synthetic Biology's recognition that IPLCs regard all components of "Mother Nature" as living entities (SCBD, 2017). In additional, in 2018, in decision 14/19, the Conference of the Parties acknowledged the need for FPIC of IPLCs in relation to the release of LMOs containing engineered gene drives (see subsection 8.1.7). Yet significant challenges remain in operationalizing participatory decision-making and the FPIC of IPLCs.

Recognizing and soliciting indigenous and traditional perspectives on synthetic biology, and coherently integrating such perspectives, is a persistent challenge. Indeed, in the context of the Māori people, there is no single Māori "perspective" on synthetic biology, but rather, there are many; thus understanding the range of views within and also across communities requires deep engagement with diverse members of potentially affected communities (Redford et al., 2019). Additionally, how IPLCs perceive nature, the unique way that they interact with it, and how this can be captured by the global regulatory governance and regulatory scheme, as well as in the risk analysis and management of impacts associated with synthetic biology, each present unique challenges that must be considered and overcome in relation to FPIC.

The challenges related to FPIC of IPLCs should be considered in the broader context of community engagement and public consultation. Useful guidance in this regard was provided in a report by the US National Academies of Sciences, Engineering, and Medicine which considered governance and public engagement as related to the developing technology of synthetic biology and gene drives, and recommended effective and tailored public engagement (National Academies of Sciences, Engineering, and Medicine, 2016b). The same report defines engagement as "*seeking and facilitating the sharing and exchange of knowledge, perspectives, and preferences between or among groups who often have differences in expertise, power, and values*". Further, it differentiates communities, stakeholders, and the public, respectively, as "*groups of people who live near enough to a potential field trial or release site that they have a tangible and immediate interest in the project*", and people with "*interests sufficient to justify engagement, but may not have geographic proximity to a potential release site*", and finally "*groups who lack the direct connection to a project that stakeholders and communities have but nonetheless have interests, concerns, hopes, fears, and values that can contribute to democratic decision making*". Additionally, the report notes that synthetic biology typically has cross-border implications and so engagement of each of these categories of people must be considered on a global scale, particularly in relation to LMOs containing engineered gene drives, given their potential risk of irreversibility once released in the wild.

Conversations have advanced concerning the importance of the engagement of communities, stakeholders, and members of the public in governing synthetic biology applications designed to affect ecosystems, such as the potential release of engineered gene drives. They have yet to result in clear, specific, or enforceable guidelines concerning FPIC of IPLCs and participatory decision-making; however, WHO recently published a revised version

of their guidance framework for testing "genetically modified mosquitoes" which requests researchers to respect the interests of those within communities hosting the test trials who may be associated with and/or affected by the research in a meaningful way (WHO, 2021). Similarly, gene drive researchers and developers have begun to pursue strategies of engagement that resemble some of the principles of FPIC (George et al., 2019). The same authors promote responsible public engagement to enable mutual learning to occur and which explicitly recognizes the rights of self-determination, which in their view, is a moral obligation situated deeply in the responsible conduct of science and which, as echoed by WHO (2021), can further engender forms of trust based on the honest intention of respecting one another's knowledge, concerns, and goals.

An example is the engagement strategy organized and funded by Target Malaria to both educate and seek the approval of communities for experiments that may eventually lead to an engineered gene drive mosquito to combat the spread of malaria.[62] This approach, however, is not without its critics, receiving accusations of "*ethics dumping*" from "*doing experiments in a foreign setting with more lax regulations*" (Bassey-Orovwuje et al., 2019). Similarly, the Mice Against Ticks project[63] has sought community consent prior to the development of a genome-edited mouse to interrupt Lyme disease transmission, and community steering committees have been formed to guide the research. The need for more robust and standardized approaches to participatory decision-making in the context of synthetic biology applications is considered further in section 11.

Reciprocally, socially informed scientific initiatives need broader support from the scientific community, funders and policymakers. Examples include the Scientific Citizenship Initiative[64] at Harvard University in the USA, which trains scientists to align their research with societal needs. The Summer Internship for Indigenous Peoples in Genomics offers genomics training that focuses on integrating indigenous cultural perspectives into gene studies. The AI Now Institute[65] at New York University (USA) has initiated a holistic approach to artificial-intelligence research that incorporates inclusion, bias and justice. Editing Nature[66] provides platforms that integrate scientific knowledge with diverse cultural world views to foster the responsible development of environmental genetic technologies. Also, Sheila Jasanoff, founding director of the Program on Science, Technology, and Society at Harvard Kennedy School, who has led calls for a "global observatory" to promote exchange across disciplinary and cultural divides on gene editing through an international network of scholars and organizations, has also suggested the approach should be used to address emerging technologies more broadly.[67]

5.2. Economic concerns

5.2.1. International trade

The Cartagena Protocol on Biosafety contributes to ensuring an adequate level of protection in the field of the safe transfer, handling and use of LMOs resulting from modern biotechnology. Of note, the Protocol establishes core procedures and a set of standards relating to the import and export (i.e. transboundary movement) of LMOs. As such, there are clear areas of linkages between the Protocol and international trade rules, in particular the WTO rules. In addition to the Agreement on Technical Barriers to Trade (TBT Agreement), the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) is relevant. Article XX of the General Agreement on Tariffs and Trade (GATT) provides for exceptions from GATT rules in order to protect health or the environment. The different fundamental objectives of the international trade and environmental regimes have led to differences in the regulatory measures taken to achieve these objectives. Strengthening the coherence of these two systems requires measures

62 https://targetmalaria.org/what-we-do/our-approach/#stakeholder-engagement.
63 https://www.media.mit.edu/projects/preventing-tick-borne-disease-by-permanently-immunizing-mice/overview/.
64 https://sci.hms.harvard.edu/.
65 https://ainowinstitute.org/.
66 https://www.editingnature.org/.
67 https://news.harvard.edu/gazette/story/2019/01/perspectives-on-gene-editing/.

to be taken at national and supranational levels to ensure that they are implemented in a mutually supportive manner, and once again, society will have a key role to play. Further, it has been recently suggested that decision makers may need formal and quantitative studies on potential economic impacts of handling, for example genome-edited products, under different regulatory scenarios. Such studies would allow them to weigh the impact of different regulatory/policymaking options on the economy (considering trade, agro-industrial innovation and productivity) (Whelan & Lema, 2017). In order to anticipate the social perception of these decisions, it may be useful to have a formal analysis of the trajectory or dynamics that the "interpretative flexibility" is taking, i.e. the various regulatory classifications (e.g. synthetic biology product, genetically modified product, conventionally-bred product, etc.) ultimately assigned to the same product in different jurisdictions (Duensing et al., 2018).

5.2.2. *Production of analogues of naturally occurring molecules*

As was reported in subsection 3.3.1, synthetic alternatives and replacements for substances or materials conventionally derived from nature are gaining ground in research and on the market. There are conservation-related motivations for instance behind the development of synthetic biology-produced alternatives that could be substitutes for products from wild species (e.g. synthetic rhino horn and synthetic horseshoe crab blood) (see section 4.4). Further, many commercial synthetic biology applications replicate naturally occurring molecules that are expensive or difficult to source outside the laboratory or produce in the laboratory using synthetic chemistry, for example the production of artificial flavours (Wellhausen & Mukunda, 2009). The main economic drivers for this appear to be as follows (United Nations Conference on Trade and Development, 2019):

(1) The establishment of reliable and economically profitable production systems that are environmentally benign in comparison with the classic production approaches based on large-scale organic chemical synthesis; and

(2) That legislation in the European Union and the USA allows compounds produced through a living organism to be labelled as "natural" rather than artificial.

The substitution of some of the natural products (i.e. naturally occurring molecules obtained from plants) can potentially ease pressures on wild or cultivated species, but it can also displace cultivation practices, often in tropical and subtropical regions. If not handled sensitively, this therefore may bring them into conflict with, or displace, those naturally sourced products which underpin the livelihoods and fragile economies of smallholder producers (ETC Group, 2016; ETC Group & Fibershed, 2018; UNCTAD, 2019). The displacement of crops cultivated by smallholder farmers is not an impact unique to synthetic biology, nor are the experiences of these farmers pre-determined. Indeed, the substitution of natural products by synthetic biology-produced versions follows a "*tradition of major technological advances that have displaced former methods of production*" (Wellhausen & Mukunda, 2009). However, the rate to which product substitution by synthetic biology applications designed to produce analogues of natural occurring molecules occurs is very much case-specific and more nuanced than originally anticipated. For example, Evolva and International Flavors and Fragrances, Inc. can market their vanillin, which is produced using synthetic biology techniques in yeast fermentation (see subsection 3.3.1(d)), as a natural product in the EU. However, as naturally sourced vanilla remains highly valued by consumers, it seems most likely that synthetic biology vanillin will compete directly with other vanillin resulting from bioconversion instead of replacing natural vanilla and its associated cultivation practices (United Nations Conference on Trade and Development 2019).

Potential adverse effects could arise though from the creation of a legal market for synthetically manufactured substitute products which would render the enforcement of illegal trade in wild-sourced products difficult or impossible (Redford et al., 2019), and which would be further exacerbated when users believe that wild-sourced products are more

efficacious or the synthetic product lacks the quality, expense and rarity (Gratwicke et al., 2008; Redford et al., 2019; Thomas-Walters et al., 2021). Further, traders may find ways to differentiate between synthetic and natural wildlife products, leading to higher prices for the natural product. Additionally, with a greater availability of synthetic products, more consumers may be attracted and seek the non-synthetic product or it could lead to greater public acceptance of natural products, such as horns (Broad & Burgess, 2016; CITES, 2018).

In the specific case of synthetic horn products, Chen and 't Sas-Rolfes (2021) considered a theoretical economic model for synthetic wildlife products and noted two opposing effects on poaching: a price effect and a laundering effect. The authors noted that as synthetic alternatives become available, the price would fall and lead to reduced poaching. In contrast, they noted that the sale of synthetic alternatives may also encourage poaching by making it easier for poachers to sell their illegal goods. However, in the case of products for which consumer demand is thought to be price-inelastic (e.g. rhino horn), their analysis suggested that legalizing the trade of synthetic substitutes could be an effective way to curb the level of poaching. In addition, biofabricated 3D-printed horn could also be used as a direct replacement for artisans, which could also reduce poaching demand (Pandika, 2017). In contrast, producers of synthetic horn products may prefer to keep prices at a level high enough that this inadvertently still encourages a significant level of poaching (Chen, 2017). Therefore, until a product is commercially available, there is a high level of uncertainty surrounding synthetic horn products.

Finally, another example is the antimalarial semi-synthetic drug artemisinin, which is a high-profile example of the trade-offs that may result from product substitutions. The shrub *Artemisia annua* has been used in China for centuries to treat a variety of illnesses, including malaria (White, 2008). OneWorldHealth, Amyris and Sanofi partnered to produce semi-synthetic artemisinin. Wellhausen and Mukunda (2009) expected semi-synthetic artemisinin (SSA) and other commercial synthetic biology applications to possibly improve health and thus the standard of living in developing countries, while simultaneously displacing labourers, exports, and the tax base of those same countries. A recent evaluation by the United Nations Conference on Trade and Development (2019) on whether SSA will eventually eliminate, or significantly reduce, the market for the natural artemisinin product concluded that it will very much depend upon whether it can compete with the natural product on the basis of price. Due to significant improvements in extraction methods from *A. annua* waste products, naturally sourced artemisinin is very cost-competitive, and so for the time being, SSA is expected to only be a supplemental source to fill gaps in production or spikes in demand.

This complex web of potential interactions is therefore adding to the challenges of assessing the potential impacts that could be associated with the use of synthetic analogues, and thus there may be the need to consider creating common rules and decision-making processes for products from synthetic or cultured DNA that maximize their benefits while minimizing any negative impacts, especially for those subject to international trade (see subsection 5.2.1).

5.3. Ethical concerns

The above examples also demonstrate how synthetic biology can raise ethical issues around harms, benefits and risks. Some risks might be deemed morally unacceptable because of the severity of harm and/or the probability of harm occurring (Schmidt et al., 2010). The distribution of potential harms and benefits related to synthetic biology products and technologies is therefore an ethical matter (Nuffield Council on Bioethics, 2012; Parens et al., 2009; Schmidt et al., 2010). What would be an equitable distribution of synthetic biology related harms and benefits, and how can that distribution be achieved? Ethical issues around harms and benefits also incorporate discussions on global justice, and the potential impacts of synthetic biology on the "technology divide" (EGE, 2009).

Questions of synthetic biology's impact on attitudes to biodiversity and conservation continue to

be asked, especially around how synthetic biology will change public perceptions of what is natural, and if it will "*challenge the ethical basis for conservation action*" (Redford et al., 2013). It has been speculated that synthetic biology could "*encourage an inaccurate model of biodiversity protection as maintaining an inventory of biological units*" (Norton, 2010). Building on this, Redford et al. (2013) noted the increasing importance of ecosystem services in valuing biodiversity and asked what will happen if ecosystems with synthesized elements are able to out-compete natural ecosystems, "*delivering more services with less biodiversity*". The "financializaton" of natural processes, which separates and quantifies environmental cycles and functions such as carbon, water, forests, fauna, and biodiversity, by turning them into units to be sold in speculative financial markets, is rejected by some as "*a violation of the sacred*" (Goodtooth, 2015). More recently, the debate about the potential use of synthetic biology with engineered gene drives have raised concerns not only about the potential impacts on biodiversity, but also ethical concerns about who will/should decide on the use of an application that could potentially spread across national borders. The scenario of a country approving the application and a neighbouring country restricting its use is feasible and raises questions about governance and ethical issues that could be also related with free, prior and informed consent (see subsection 7.1.2).

Synthetic biology is seen by some to raise ethical issues related to intellectual property (IP) rights; others consider synthetic biology as a way to avoid ethical challenges to "patenting life" or to bypass benefit-sharing obligations associated with the utilization of genetic resources. Considerations of justice include the distribution of material and non-material goods. The application of intellectual property rights to synthetic biology, such as patents on DNA sequences or organisms resulting from synthetic biology, could restrict the global distribution of products and knowledge (ENCH, 2010; ICSWGSB, 2011; Schmidt, 2009). Civil society groups strongly critique the way that IP regimes have been used in agricultural biotechnology to concentrate power with a few corporations, and they see similar patterns of use occurring in synthetic biology (ICSWGSB, 2011; ETC Group, 2010; Friends of the Earth, 2010). Using synthetic biology to design and synthesize DNA sequences is also, however, seen by some as a way to avoid ethical and legal challenges – particularly those related to patenting the sequence information of naturally occurring DNA (Torrance, 2010). The potential to synthesize DNA sequences and downstream metabolic intermediates and pathways also raises contrasting views regarding equitable access and benefit-sharing associated with the utilization of genetic resources as is evident in the discussions in several international fora concerning "digital sequence information" which have been informed by deliberations on synthetic biology within the Convention (see subsection 8.1.5 below).

Ethical considerations of biodiversity and of how people relate to biodiversity are also recognized as important in the context of the Convention. For example, at its tenth meeting, the Conference of the Parties adopted the *Tkarihwaié:ri Code of Ethical Conduct to Ensure Respect for the Cultural and Intellectual Heritage of Indigenous and Local Communities* (decision X/42). The *Tkarihwaié:ri Code* identifies general ethical principles, including prior informed consent and/or approval and involvement of indigenous and local communities; the fair and equitable sharing of benefits with them; and the precautionary approach, including involvement of relevant indigenous and local communities and the use of local criteria and indicators in the prediction and assessment of potential harms to biodiversity (decision X/42, annex, section 2(A)).

Ethicists have actively engaged with the new tools and techniques of synthetic biology for more than 20 years (Cho et al., 1999), with the Nuffield Council on Bioethics (2016) concluding that the ethical debate on synthetic biology is further exacerbated by the novel mode of action, increased accessibility and speed of use and uptake associated with the technologies and platforms provided.

Common considerations have for instance included the ethical debate on whether to ban publications of dual-use science discoveries and whether synthetic

biologists are "playing God" (Boldt & Müller, 2008; Douglas & Savulescu, 2010; Kaebnick, 2009; The Royal Academy of Engineering, 2009). However, for some, "playing God" may not be regarded as problematical. One could argue that humans are the God species and should take control over natural processes in order to achieve human flourishing on this planet (Bovenkerk & Nijland, 2017). Thus, the role of human intervention in nature and natural processes, including this idea of *naturalness*, have been raised as there could be a greater need to understand our values of nature, goals for conservation and the promise of biotechnology (Graeff et al., 2019). With the advent of new technologies, the biophysical influence of humans on nature could be more profound, having implications for biological evolution by controlling whole ecosystems and species (Graeff et al., 2019; Kaebnick, 2009). For example, editing a gene which has evolved over thousands of years could be viewed as a disruption to natural homeostasis (Šutković et al., 2020). Further, in the case of modifying genomes, the idea of *integrity* could be challenged with our understanding of how a genome constitutes an organism (Bovenkerk & Nijland, 2017). Another common consideration centres around the possibilities of either using this technology potentially irresponsibly and causing harm, or not using it at all, which could also prove damaging to humans, our welfare, and our planet (Kofler et al., 2018).

With regard to animal welfare, the techniques and technologies of synthetic biology have the potential to alleviate animal suffering in agricultural settings (Graeff et al., 2019). Some could view the application of synthetic biology techniques as analogous to selective breeding, especially in cases where the species-specific function is not hampered (Bovenkerk & Nijland, 2017). On the other hand, others may consider it to be an affront to an animal's dignity or could prevent the animal from living according to its instincts, which may or may not be relevant given our understanding of the human values of self-determination and being a moral agent. Additional concerns could also be related to the sustained use of animals in research as a result of an increased interest in modifying them, which may contribute to more suffering, especially in the context of off-target effects that may lead to birth defects or post-natal death, or that perpetuate poor animal management in intensive farming operations (Cotter & Perls, 2019; Graeff et al., 2019). Moreover, practices to modify animals may be a further exertion of human control over animals, which may be morally unacceptable if animals are viewed only as objects for human use (Ayanoğlu et al., 2020). In contrast, synthetic biology applications, for example based on cell-free systems, minimal cells, and differentiated tissues and organoids, are being developed as a means to reduce the need for animals in research (El Karoui et al., 2019).

In the field of conservation biology, some practitioners have expressed hope for a convergence between the traditional past-looking conservation mindset and the forward-looking optimism of synthetic biology, with speculation that it could contribute to saving endangered species and even reviving and restoring extinct species (Redford et al., 2013, 2014). Underlying this hope is recognition that new approaches and strategies are needed to address biodiversity loss that continues despite the application of conservation efforts. The optimism expressed by some is not shared by all members of the conservation community, with some expressing deep concern that applications of synthetic biology may serve as "Trojan horses" for other "more questionable" applications (Keiper & Atanassova, 2020). Further, the application and efficacy of proposed synthetic biology approaches in the field are likely to encounter multiple hurdles which will require further development to overcome, or may even prove to be intractable barriers to useful application (Redford et al., 2019). Redford et al. (2019) go on to make a plea for the policy debate to be grounded in evidence, emphasizing that conservation practice "*needs to be rigorous and defensible, building on impartial standards that are free from ideology or political bias yet transparent in its advocacy for the natural world*". Moreover, Boldt (2018) argues for remaining realistic with regard to synthetic biology hopes and promises, for keeping track of the whole field of possible technological and social solutions to societal problems, and for embedding synthetic biology applications in a social context that allows long-term safe and

just use. There therefore remains a large scope for society to be further involved in formative discussions concerning the acceptability or otherwise, and thus consequently the regulation, of synthetic biology applications and products.

5.4. Concerns arising from dual use

Bioterrorism, biological warfare and the construction of novel organisms designed to be hostile to human interests can all potentially be achieved through the malicious use of many different technologies in the life sciences, including those underpinning synthetic biology, with the term "dual use" used in discussions when both beneficial and harmful effects can be the outcome(s) of using the same technology. An example of a potential harmful dual use would be bioterrorists creating new pathogenic strains or organisms resistant to existing defences. Currently, it is possible to enhance the virulence of known pathogens with new traits that can contribute to their competence and resistance to existing treatments. For example, a novel type of avian flu virus with enhanced infectivity in mammalian animals may be created, and the H5N1 influenza virus can be modified to evolve into a dangerous human virus (Herfst et al., 2012). Mukunda et al. (2009) predicted that biological weapons customized to attack specific groups were highly likely to be developed in the long term (10 or more years), i.e. by the time of this technical series update; however, there are no known reports of such weapon development to date. Although microbes are usually the main platform for the development of applications with malicious intent, plants are not immune to such approaches. It has been recently suggested that criminals may exploit modern genome editing technologies to subject market LMOs to clandestine manipulation (or the malicious insertion of genetic modifications into ostensibly unmodified plants), raising the prospect not only of direct harm, but of the more likely effects in generating public concern, reputational harm of agricultural biotechnology companies, lawsuits, and increased import bans of certain plants or their derived products (Mueller, 2019). It has been further suggested that when (mis-)used, especially in combination with newer technologies such as engineered gene drives, virus-mediated methods, or *in vitro* evolution techniques, the effectiveness of current authentication and surveillance protocols may be overridden. Unfortunately, it is by no means clear that such abuses could be entirely eliminated, any more than they can be for other "dual use" technologies. Discussions at the international level have resulted in the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction (the "Biological Weapons Convention"; BWC) and are explored more fully in subsection 9.3.1(b).

One such example is the "Insect Allies" programme funded by DARPA (see subsection 3.2.3(d)). The project aims to develop countermeasures against natural threats, as well as countermeasures against State and non-State actors, by releasing insects to disperse modified viruses to rapidly introduce traits to crop plants (DARPA, 2016). However, Reeves et al. (2018) considered that the knowledge potentially gained by the project appears to be limited in its capacity to enhance agriculture in the USA or respond to national emergencies. The use of insect dispersal was of particular concern as the modified viral agents could also be spread via spraying without the need for insects. The authors noted that spraying equipment would be simpler to scale up in times of emergency than the difficult process of increasing insect production systems. Noting omission of regulation from the press releases, the lack of robust explanations and publicly available analyses on trade and biosafety, the authors questioned if the goal of the project was to develop novel bioweapons, which would violate the Biological Weapons Convention (subsection 9.3.1(b)), and voiced concern that this may lead to other nations developing their own bioweapons. Further, it was noted that funding for projects reflected an applied nature due to the explicit discounting of projects based on model plant organisms, such as tobacco or *A. thaliana*, and focusing on crops of agricultural importance, such as maize, rice, cassava, cowpea, wheat, potato, etc. (DARPA, 2016). Thus, it could be theorized that such applications could have food security implications if deployed (Reeves et al., 2018).

Beyond actions potentially coordinated by governments or organized groups, there are additional concerns over the potential emergence of a "biohacker" culture in which lone individuals could theoretically develop dangerous organisms, analogous to the creation of computer viruses. The basic technologies for systematic genetic modification of organisms are widely available and are becoming cheaper, although it is easy to underestimate the degree of technical proficiency, experience and resources needed to make effective use of them. Many researchers in the field anticipate that the real harms that might be inflicted by such "hacker" activities are probably small; they nonetheless warrant careful consideration (Mueller, 2019). Scientists, their host institutions and funding bodies should therefore seriously consider whether their research could be misused, and in cases where it could, should implement and clearly communicate measures to reduce the likelihood of misuse and its consequences (El Karoui et al., 2019). Further, it is difficult to see how they might be prevented – the question is therefore more about law enforcement than scientific protocol. In this regard, more than 40 countries have joined the "Australia Group",[68] an informal forum of countries which uses licensing measures to ensure that exports of certain chemicals, biological agents, and dual-use chemical and biological manufacturing facilities and equipment do not contribute to the spread of chemical and biological weapons. Included within their Common Control Lists[69] are "Genetic Elements and Genetically-modified Organisms",[70] which includes genetic elements that code for any gene(s) "*specific to any listed virus, bacterium or fungus, and which in itself or through its transcribed or translated products represents a significant hazard to human, animal or plant health, or could endow or enhance pathogenicity; or any listed toxins or their sub-units*" (see also subsection 9.3.1(b)).

As of 2019, no country requires the companies that sell synthetic DNA to prevent "questionable parties" from acquiring materials (Koblentz, 2020). However, the majority of double-stranded DNA sequences are made to order by commercial providers who are members of a group, known as the International Gene Synthesis Consortium (IGSC; see also subsection 7.3.3),[71] which implements a Harmonized Screening Protocol to voluntarily screen all orders in alignment with guidance[72] from the US Department of Health and Human Services. While there is not a single DNA screening algorithm used by all IGSC members, DNA-screening software typically aligns a query sequence and 200 bp subsequences to a reference database of biological toxins and select agent genomes, genes, or proteins as a means of addressing biosecurity concerns associated with the potential misuse of their products to bypass existing regulatory controls (Elworth et al., 2020). Challenges have been identified in the implementation of the current screening process since its inception in 2010, to the extent that there is an open call for public comments on whether and, if so, how the guidance should be modified to address new and emerging challenges posed by advances in this area (USA Health and Human Services Department, 2020). Further, there have also been suggestions to increase cyber-biosecurity for DNA synthesis and laboratories. Depending upon screening implementation, some DNA sequence obfuscation[73] techniques may permit

68 The States participating in the Australia Group are parties to the Chemical Weapons Convention and the Biological Weapons Convention. Coordination of national export control measures assists Australia Group participants to fulfil their obligations under those conventions. The Australia Group meets annually to discuss ways of increasing the effectiveness of participating countries' national export licensing measures to prevent potential proliferators from obtaining materials for chemical or biological weapons programmes. Meetings of the Australia Group have discussed synthetic biology since 2007. See www.australiagroup.net.

69 https://www.dfat.gov.au/publications/minisite/theaustraliagroupnet/site/en/controllists.html.

70 In this case, genetically modified organisms include organisms in which the nucleic acid sequences have been created or altered by deliberate molecular manipulation, whereas genetic elements include, *inter alia*: chromosomes, genomes, plasmids, transposons, vectors, and inactivated organisms containing recoverable nucleic acid fragments, whether genetically modified or unmodified, or chemically synthesized in whole or in part.

71 https://genesynthesisconsortium.org.

72 Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA, https://www.phe.gov/preparedness/legal/guidance/syndna/documents/syndna-guidance.pdf.

73 Obfuscation is when code is purposely complicated to conceal what it performs.

unauthorized access to controlled DNA sequences. In a proof-of-concept experiment, researchers were able to successfully order a toxic peptide from a DNA synthesis company (Puzis et al., 2020). Notably, therefore, no government or private policy can ever achieve perfect compliance, even in traditional scientific settings. Striving for perfect compliance may come with substantial burdens, including slowing the development of new technologies and increasing expenditures on enforcement resources. Given this, both public and private policymakers and regulators may wish to tailor existing regulatory mechanisms to mitigate genetic biohacking risks in a manner that does not unnecessarily reduce technological potential (Zettler et al., 2019).

Another such challenge is that, as these technologies become ever more accessible, gene sequences can be procured by means other than through companies capable of sophisticated customer screening procedures. It may be that the threat is much greater from State-sponsored terrorism (for which DNA synthesis would be hard to control or monitor) than from amateur activities. However, it is important not to underestimate the difficulty of moving from research in a laboratory, let alone a "biohacker" garage, to a functioning product that can be disseminated widely and rapidly (e.g. by aerosol). Incorporating synthetic biology techniques into research on biology does not mean that the resulting products can be easily developed as if they were just another piece of hardware or software.

The dissemination of the technology, knowledge and capabilities involved in synthetic biology, both within and beyond the professional biotechnology community, will have two (potentially overlapping) strands (see International Risk Governance Council, 2009; SCBD, 2015; National Academies of Sciences, Engineering, and Medicine, 2016b; InterAcademy Partnership, 2018):

(1) Professional groups such as engineers and computer scientists, educated in disciplines that do not routinely entail formal training in biosafety, may acquire these capabilities. In consequence, there needs to be a dialogue between all relevant researchers and regulators on what responsible conduct might entail in this field, and education about the risks of, and guidance on best practice for, biosafety principles and practices applicable to synthetic biology. A review of biosafety standards should also be conducted to identify differences between standards and actual laboratory practices.

(2) Dissemination may extend beyond academic and professional circles as biological engineering becomes more accessible. This may include less responsible individuals and organizations. Legitimate researchers can help governments and regulators to find ways to prevent other actors from using the technology for illicit purposes. An appropriate balance also needs to be found between top-down command and control and bottom-up education and awareness initiatives, including the fostering of a culture of responsibility and the de-glamorization of the kind of antisocial activities already evident in the creation of computer viruses.

In a positive light, synthetic biology could provide tools for responding to biosecurity risks arising from harmful dual use. The US Presidential Commission for the Study of Bioethical Issues (PCSBI) (2010) claims it is "*easy to anticipate potential benefits*" of synthetic biology to biosecurity, such as identifying biological agents of concern and countering biosecurity threats. Synthetic biologist Drew Endy urges that synthetic biology be understood in terms of its "*net contribution to risk exposure and not only risk creation*" (Endy, 2005). Thus, although synthetic biology can be used to create threats, synthetic biology can also be used for defence, such as improved surveillance to detect pathogenic agents, accelerated vaccine production, and provision of therapies for some pathogens (Endy, 2005; Mukunda et al., 2009). This has been exemplified by the world's reaction to the current COVID-19 pandemic, where a novel coronavirus was detected, sequenced, and various vaccine strategies developed, resulting in numerous vaccine candidates successfully passing the three stages of clinical trials and obtaining regulatory approval, and vaccinations programmes

begun around the world – all within a period of 12 months; a remarkably short timeframe never seen before (N. Zhang et al., 2020).

6. General biosafety concerns associated with synthetic biology applications

While sections 4 and 5 provided examples on the potential impacts (positive or negative) associated with specific synthetic biology applications, this section focuses on general biosafety concerns related to the accidental or intentional release of organisms resulting from synthetic biology. These concerns and the way they may be addressed under biosafety systems will vary and depend on the type of organism and trait, intended use and receiving environment. The suitability of existing risk assessment methodologies as well as potential management strategies are also discussed. The section, however, does not intend to be a comprehensive guide or list of issues to be considered under any specific assessment, as every potential analysis will have to be done on a case-by-case basis and in accordance with national and international regulations.

6.1. Adequacy of risk assessment methodologies

The wide range of synthetic biology applications under development (section 3) exemplifies the different types and characteristics of such synthetic biology organisms and products. While some might present little or no complexity and novelty compared to those produced by other methods or those coming for example from genetic engineering (i.e. LMOs), some might represent a completely new organism, for instance those from genome-level engineering. Therefore, the adequacy of current methodologies for the environmental risk assessment of synthetic biology products might depend on the degree of novelty and complexity observed (Wikmark et al., 2016). Different methods and techniques of synthetic biology may need different forms and levels of oversight. Thus, any new risk assessment framework, cost-benefit analyses and regulations must flexibly encompass different applications, uses and products (ETC Group, 2012), and treat each application on a case-by-case basis. An additional element for consideration in this regard may also be the appropriateness of a product-based or process-based approach to inform the risk assessment process (Academy of Science of South Africa, 2016).

Any requests for synthetic biology applications to be used in unmanaged or managed settings will likely be evaluated within a risk-based regulatory decision-making process. This process will be influenced by, for instance, ethical, socio-cultural, epidemiological, ecological and economic considerations (see previous two sections), and the process should include mechanisms that facilitate the effective engagement of stakeholders and help integrate these considerations within the overall decision-making process (Hayes et al., 2018) (see section 7).

The overall aim of a risk assessment is to identify, characterize and evaluate risks to the environment and to the health and safety of people. Essentially, to do so, a potential risk is identified by considering what could go wrong and how harm might occur, while the identified risk is characterized by considering how serious the harm could be (consequences) relative to the conventional counterpart/suitable comparator and how likely that harm could occur (likelihood) within the context of the case and its intended use (Gray, 2012). This is consistent with a long-standing understanding in other domains that risk is the combination of the magnitude of the consequences of a hazard with the likelihood that the consequences will occur. By integrating consequences and likelihood, the level of risk can be evaluated, compared to those presented by the comparator, and the need for any measures to reduce it considered when pertinent. Risks are characterized by testing specific hypotheses on the probability that harm will occur and the severity of the harm if it does occur. This process is framed by a problem formulation approach that articulates relevant policy goals, determines criteria for assessing risks, and devises tests of risk hypotheses that address those criteria (Connolly et al., 2021; Craig et al., 2017; Devos et al., 2019). As noted earlier, however, non-scientific criteria should be included

in the process of judging the acceptability, or otherwise, of any characterized risk (see subsection 5.1.1).

Although the risk assessment methodologies may differ between countries and their regulatory authorities, the risk assessment is a process based on available scientific evidence that, as mentioned before, is aimed at informing the decision-making process. For the specific case of synthetic biology organisms that fall within the definition of a LMOs as per the Cartagena Protocol on Biosafety, its Annex III (SCBD, 2000) (section 8.2 above) sets out the general principles of a science-based risk assessment and general methodology, including "points to consider" that may be extended/adapted to some applications resulting from synthetic biology.

Some such applications are challenging existing regulatory systems due to the need to address novel risks and impacts, the levels of uncertainty and currently untested mechanisms for observation and monitoring (Duensing et al., 2018). Together, these are compounded by the ever-increasing pace of development of these technologies (Fidelman et al., 2019; Linkov et al., 2018). For example, microbes present a particular case in point due to their amenability to being subjected to a wider range of synthetic biology techniques. "*Although no novel hazards have been identified for current and near future synthetic biology microbes, the efficacy by which they interact with their biotic and abiotic environment may differ, and this may lead to increased exposure and therefore may result in higher risk*" (More et al., 2020). Instead, those synthetic biology microorganisms likely to be developed beyond the next 10 years for deliberate release into semi-managed, managed and urban settings, especially those such as minimal cells, protocells and xenobionts, the same authors conjectured that these microorganisms *"may lead to novel hazards … e.g. due to 1) new-to-nature organisms/products/constituents possibly with poorly understood interactions with their biotic and abiotic environment, 2) xeno-proteins with new enzymatic properties, i.e. modified substrate specificity or higher environmental robustness, and so opening new environmental niches, and 3) substantial reductions of the genome which could lead to unexpected interactions with other organisms (e.g. those that lead to evasion of the immune system)*". Thus, More et al. (2020) conclude that risk assessment guidance will require updating to take into account all microorganisms (e.g. microalgae, viruses, xenobionts), their relevant exposure routes and receiving environments, and to consider other risk assessment approaches that are not solely based on the comparative approach for new-to-nature components. To date, regulatory practices have relied upon risk assessment to quantify the risks of materials and technologies and upon management to restrict risks to acceptable levels, typically by limiting exposure of humans and environmental receptors (Linkov et al., 2018).

In the following subsections, risk assessment considerations are presented for applications intended for release into semi-managed, managed and urban settings from three synthetic biology supporting technologies that have received regulatory attention to date and their relevance in international discussions.

6.1.1. Engineered gene drives

As for other products of biotechnology, regulators are expected to consider, on a case-by-case basis, the potential risks and benefits from any new approach to control for instance IAS and pests (see section 4.1) compared with those from currently available methods. Some regulatory agencies are in the process of reviewing or have already reviewed their procedures for research with LMOs containing engineered gene drives in containment and acknowledge that the general principles and methodology for risk assessment and management, experiences from LMO risk assessment, as well as knowledge from fields such as biocontrol agents and invasive alien species, will all be relevant to performing risk assessments of LMOs containing engineered gene drives (Australian Academy of Science, 2017; Haut Conseil des Biotechnologies, 2017; Naegeli et al., 2020; National Academies of Sciences, Engineering, and Medicine, 2016b; Smets & Rüdelsheim, 2020; van der Vlugt et al., 2018). Challenges that are anticipated when performing environmental releases of such organisms are mainly related to the targeting of wild populations and may be irreversible (depending upon the engineered gene drive in question),

and thus the stepwise approach to environmental releases, as practiced with other types of LMOs, may require adaptation (Keiper & Atanassova, 2020). This presents new challenges for risk assessment, because for the first time we are faced with a technology whose potential ecological and health impacts cannot be adequately assessed without first deploying it (Sirinathsinghji, 2019). However, for the risk assessment of mosquitoes containing engineered gene drives, experience with releases of biological control agents, including those developed using earlier genetic engineering technologies, may provide useful precedents and insights into these challenges and how any potential transboundary movement may be managed (WHO, 2021).

Recognizing that a range of engineered gene drives with different dispersal ranges have and continue to be developed (see section 4.1), it has been reported that for those engineered gene drives designed to "*suppress or enhance a species population at a rate that is faster than natural ecological processes or evolutionary rates*" the definition of additional pathways to risk assessment endpoints may be required (National Academies of Sciences, Engineering, and Medicine, 2017). Work on this area has recently begun to emerge, using problem formulation to identify and characterize 46 plausible pathways to potential harm across 4 major policy areas (biodiversity, water quality, human health, animal health) for a specific population suppression engineered gene drive in *Anopheles gambiae* mosquitoes in West Africa (Connolly et al., 2021). This study may be used to inform the subsequent steps of hazard and exposure characterization in the environmental risk assessment of this application. Further, the European Food Safety Authority (EFSA) has noted that the temporal and spatial scope of gene drive-engineered insects will be case-specific and that once released may preclude testing by observation at such scales. As such, they noted that EU regulations concerning the molecular characterization, environmental risk assessment and post-market environmental monitoring specifically of gene drive-engineered insects are insufficient and thus want further guidance to be developed which builds upon existing approaches (Naegeli et al., 2020). It has also been suggested that an additional "spatio-temporal controllability" step be included in the risk assessment process of these organisms (Then, 2020). Similar sentiments, and others, were discussed by the AHTEG on Risk Assessment under the Cartagena Protocol on Biosafety, and the AHTEG has recommended the development of further guidance on applications featuring engineered gene drive systems (SCBD, 2020e). Although it recognized that existing risk assessment methodologies may still be applicable for such organisms, the group indicated that specific technical or methodological challenges require further attention. These include a lack of data to inform the risk assessment process; the limited applicability of some aspects of risk assessment methodologies to LMOs containing engineered gene drives (such as challenges to the comparative risk assessment framework and monitoring methods); a lack of guidance on how to assess uncertainty; a lack of validated modelling tools; and a lack of experience or capacity.

The AHTEG also recognized that solutions to the challenges posed by LMOs with engineered gene drives will entail reconsideration of risk assessment and monitoring methods, as well as making more widely available the necessary expertise, training and resources required and the participation of indigenous peoples and local communities. Due to the complexity and diversity of engineered gene drives available and the array of potential case-by-case interactions of the host organisms with the environment, questions have been raised concerning whether risk assessment could result in sufficiently reliable conclusions (Dolezel et al., 2020). Further, the risk assessment of LMOs containing engineered gene drives will also require the development of new tools to complement established methodologies. As is typical of current risk assessments, these will include the use of models, in this case to help predict the ecological consequences of released LMOs containing engineered gene drives. Unlike non-engineered gene drive organisms which can be confined in time and space during small-scale tests, thereby facilitating the generation of data in those tests of relevance to large-scale releases, the potential of LMOs containing engineered gene drives to spread over large areas and landscapes (depending upon the type of

drive),[74] even from a limited release or well-isolated trials, means that risk assessors will need to consider models and forecasts in their assessments. However, as the development of LMOs containing engineered gene drives nears potential release, further ecological work, for example the characterization of density dependencies, seasonality and spatial heterogeneities of the host mosquito populations, will be essential to enhance model predictions and better understand the systems under assessment (Sánchez et al., 2020). Such enhancement will prove challenging, especially in terms of obtaining, validating and calibrating modelling data before an environmental release (SCBD, 2020c).

6.1.2. Genome editing

Discussions on how to assess and regulate genome-edited plants essentially revolve around two approaches: those that consider (certain types of) genome-edited plants to be of low or negligible risks and those that highlight uncertainties and knowledge gaps (Schiemann et al., 2020). The latter captures concerns about, for instance, genome editing allowing for modifications that would not otherwise naturally arise (African Centre for Biodiversity, 2020; Heinemann et al., 2021).

When it comes to risk assessment considerations associated with potential unintended off-target effects in genome-edited plants, they need to be viewed in the context of the well-documented dynamic nature and plasticity of plant genomes. The potential for unintended changes in the genome is not a unique feature of genome editing where any potential imprecision is expected to be significantly lower than the rates of spontaneous mutations or classical mutagenesis (Duensing et al., 2018; Naegeli et al., 2021; Scientific Advice Mechanism, 2017). It has also been noted that the precision of genome editing could lower the frequency of some unintended events (Lassoued et al., 2019). However, for staple food crops with large and complex genomes, such as wheat, barley or maize, off-target editing is more likely to occur (Agapito-Tenfen, 2016). Although recent research in the medical field, principally on mouse and human cells, has identified a range of unintended effects from the use of genome editing (e.g. Brinkman et al., 2018; Kosicki et al., 2018; Leibowitz et al., 2021), Schnell et al. (2015) note that in plants, the relationship between genotype and phenotype is complex and is also tempered by the environment; thus the buffering capabilities of plant genomes and the quality control systems in plant cells will prevent many genetic changes (e.g. introduced spontaneously or through conventional breeding or genetic engineering) from giving rise to discernible changes in the plant phenotype.

It has been argued that the current approach to risk assessment is not designed to detect unintended consequences that may arise from employing some genome editing techniques (Christ et al., 2018). In response to this, there have been proposals that untargeted metabolomics could be part of a routine protocol assessing genome-edited crops (Lassoued et al., 2019). In a separate proposal, risk assessments could be tailored to the expected levels of uncertainty. For example, a "risk assessment light" could be implemented for cases with minimal changes and familiarity with the particular trait or plant of use (Schiemann et al., 2020). Several traits in plants (e.g. herbicide resistance, modified composition) that are developed with the utilization of new genetic modification techniques are already provided in LM crops and experience has been acquired with the related risk assessments. Other traits being developed in plants, however, are novel, meaning they are not present in currently cultivated agricultural plants, and their underlying physiological mechanisms are not yet sufficiently elucidated. It has been noted, though, that the characteristics of some genome editing applications, e.g. the small extent of genomic sequence change and their higher targeting efficiency, i.e. precision, cannot be considered an indication of safety *per se*, especially in relation to novel traits (Eckerstorfer et al., 2019). Further, it has been cautioned that when it comes to assessing mutations introduced by genome editing, risk assessment approaches should also address impacts derived from them being driven by human activity

74 Strategies to limit the spread of engineered gene drives are explored in subsection 6.2.3.

that result in their establishment and spread in the environment at a rate far quicker than evolution (Heinemann et al., 2021).

As discussions about the safety of genome-edited organisms continue and information becomes available, countries are starting to discuss how best to assess any potential risks that may come from their use. For instance, the EFSA GMO Panel considered that its existing guidance for risk assessment of food and feed from genetically modified plants and the guidance on the environmental risk assessment of the same are sufficient, but are only partially applicable to plants generated via SDN-1, SDN-2 or ODM. They went further to state that the information requirements of those guidance documents that are linked to the presence of exogenous DNA are not relevant for the risk assessment of plants developed via SDN-1, SDN-2 or ODM approaches if the genome of the final product does not contain exogenous DNA (Naegeli et al., 2020). Obviously, any identified remaining exogenous DNA would have to be assessed, especially if it discernibly affected the plant phenotype (Schnell et al., 2015). In a separate evaluation of the same EFSA guidance, this time making use of hypothetical case studies based on two synthetic biology categories, namely genetic part libraries and methods, and DNA synthesis and genome editing, the EFSA GMO Panel (2021) concluded, *inter alia*, that (a) when compared to established techniques of genetic modification, "*no potential novel hazards were identified nor novel potential risks in terms of impact on humans, animals and the environment*", and (b) their current requirements "*are adequate and sufficient for the risk assessment of such cases, although not always applicable*". In a similar vein, the US National Academies of Sciences, Engineering, and Medicine (2017) indicated that for products such as "next-generation" LM crops, it was not anticipated that risk assessment endpoints would be different from previously assessed LM crops. However, in a horizon scanning exercise undertaken to identify synthetic biology developments in the agri-food sectors likely to enter the market, for those expected to enter beyond the next decade, the EFSA GMO Panel (2021) acknowledged that their guidelines may need to be adjusted to ensure that they are "*adequate and sufficient*", and risk assessment approaches may be needed that "*do not rely on a history of safe use and the current comparative approach*". Although not unique to genome editing, the French Scientific Committee of the High Council for Biotechnology identified the following three points to consider in terms of potential risks related to environment and health, as compared to conventional breeding: (1) technical unintended effects related to effector persistence as well as risks associated with off-target modifications or other unintended genome modifications, (2) risks arising from the desired trait and its novelty in the plant, and (3) risks associated with the potential modification of plant breeding practices, owing to efficacy and technical ease of use of genome editing, be it for single traits or for combined modifications (multiplex genome editing) (Troadec & Pagès, 2019).

In the context of animals, it was suggested that risk assessment methodologies similar to those used to assess plants could be applied to the case of animals (Fears & ter Meulen, 2017). As is the case for plants, it could be anticipated that genome editing techniques applied to animals may also produce unintended (off-target) changes in addition to the intended genomic edition itself (Kawall et al., 2020). Similarly, in the cases of SDN-1, SDN-2 and ODM, the changes could be equivalent to changes expected from classical breeding and thus may not pose unique challenges (Jones, 2015; D. Zhang et al., 2020). However, a lack of scientific data on engineered animals, how animal systems respond to genome editing and complicated genetics (e.g. pleiotropy, alternative splicing) could complicate the perception and evaluation of risk (Cotter & Perls, 2019; Eriksson et al., 2018).

6.1.3. RNA-based technologies

Plants produced using RNA-based technologies e.g. the Flavr Savr™ tomato in 1994 (Krieger et al., 2008) and the Rainbow papaya in 1998, resistant to *Papaya ringspot virus* (Gonsalves et al., 2010), both based on antisense technology (section 1.6), were some of the first to undergo risk assessments with features similar to those later outlined in Annex III to the Cartagena Protocol on Biosafety. As

plant varieties obtained using RNAi mechanisms continue to receive regulatory approval for cultivation (e.g. plum tree resistant to *Plum pox virus* [PPV] in the USA; common bean resistant to *Bean golden mosaic virus* in Brazil; SmartStax™ maize with multiple resistance traits, including dsRNA against *Diabrotica virgifera virgifera* in the USA and Canada; cassava resistant to *Cassava brown streak virus* and *Ugandan cassava brown streak virus* in Nigeria [Arpaia et al., 2020]), different points of view have emerged concerning the approaches used to assess their potential impacts. While some regulators have considered RNAi-based GM plants to be no different from any other GM plant, others have acknowledged that they might affect their present approach for risk assessment (EFSA, 2014; Heinemann et al., 2013). It has been proposed that risk assessment strategies followed for current GM plants, and which are based on the comparative analysis of the molecular, compositional, and agronomic/phenotypic characteristics of the GM plant and its conventional counterpart, remain applicable and adequate for the evaluation of RNAi-based plants (Casacuberta et al., 2015). However, it has also been noted that the risk assessment of RNAi-based plants presents some peculiarities compared with that of currently commercialized GM crops, especially as data related to newly expressed proteins, protein equivalence and codon optimization are irrelevant for this inserted DNA as long as no part is translated into protein (Arpaia et al., 2020; Casacuberta et al., 2015). Conversely, it has been suggested that the decreased expression of a target gene may have safety implications in particular cases (e.g. if a substrate of a silenced enzyme accumulates to toxic levels) and thus should be fully assessed if identified (Casacuberta et al., 2015).

Regarding the transmission of RNA silencing from one species to another, e.g. via consuming a plant produced by RNA-based technology, Paces et al. (2017) stated that species which can absorb long dsRNA and have systemic RNAi are more prone to exhibiting specific silencing effects as well as off-target effects. However, significant off-target effects will require specific conditions in terms of stoichiometry between the small RNAs and their targets, which are unlikely to be met upon RNAi induction with long dsRNA. For those plants produced by RNA-based technology which specifically target invertebrate species, Christiaens et al. (2018) reported that knowledge on issues such as exposure, specificity, off-target effects, sequence similarities and bioinformatics remained very limited, due to only a few such plants having been developed and comprehensively studied. Brazil, New Zealand, and Australia have each approved RNAi-based GM plant events for environmental and food/feed commercialization based on risk hypotheses pertinent to their jurisdictions and without any changes or adaptations in their risk assessment procedure. This is a clear example of how different regulators perceive novelty and how they decide to act (Wikmark et al., 2016).

For RNAi induced by a spray or topical application, it was noted that dsRNA is a naturally occurring molecule that is readily degraded in nature and biological systems, therefore specific formulations to ensure its stability and effective delivery to targets will be required on a case-by-case basis (Taning et al., 2020). Such products are a novel type of biological protection "biopesticide" and it is important that safety assessments for plant protection products be adapted to allow introductions of this technology. Existing plant protection product risk assessment approaches can be reliably used to evaluate dsRNA-based products for topical application, with adaptations only required on a case-by-case basis where additional research might be necessary to assess risk (Mezzetti et al., 2020).

The evaluation of the potential risk associated with the silencing of an off-target gene is specific to RNAi technology. When used in spray formulations, Werner et al. (2020) have reported that shorter target sequences, which are also specifically selected to produce siRNAs with a minimal potential to silence unintended targets, could greatly reduce off-target effects. Therefore, they have suggested using minimal-length dsRNA sequences carefully selected based on known design criteria requirements. Another possible way to achieve high silencing efficiencies while retaining high

target specificity (less off-target effects) could be the use of dsRNAs repeating a shorter tool-designed sequence several times (Werner et al. 2020). Additionally, if less conserved regions of the mRNA are targeted, homology could be limited between sequences and therefore decrease the potential for off-targets effects (Fletcher et al., 2020). At the whole organism level, the carrier to which the RNA molecules are bound or the formulation in which they are applied will be of significant importance in the determination of potential risk to unintended organisms (non-target organisms), as each will not only affect the level at which non-target organisms will be exposed, i.e. the stability and distribution of the active compound in the environment and in the target organism, but also the extent of the RNAi response (Romeis & Widmer, 2020). Further, regulators in the USA and the EU have both expressed concern about exposure routes and how testing requirements may change with different formulations. Thus, it was proposed that it may be necessary to test target organisms at various life stages due to differential sensitivities to RNAi, and protocols for addressing hazards of dsRNA-based products will require revision compared to those for conventional pesticides because of the longer time period necessary for dsRNA-based products to display efficacy (Mendelsohn et al., 2020).

While new research and bioinformatic analyses investigate the potential environmental impacts for RNAi technologies, several considerations have yet to be addressed that may impact the evaluation of risk for these applications if deemed to be significant. These include:

- *The availability of genomic and transcriptomic sequence data sets for organisms*. Off-target effects may not be predicted if sequence data is not available (Fletcher et al., 2020);

- *The tolerance of sequence mismatches between the designed RNA molecules*. In some cases, it has been noted that mismatches in specific locations or in sequences without perfect (100%) complementarity may still elicit a silencing response (Arpaia et al., 2020; Chen et al., 2021);

- *The formulation and chemistry of dsRNA products*. In current formulations (i.e. naked dsRNA molecules), it has been observed that dsRNAs are rapidly degraded in soils, on leaves and in aquatic environments, likely due to microbial metabolism, environmental nucleases and/or ultraviolet radiation (Bachman et al., 2020). However, altered formulations (e.g. clay nanosheets, chemical modifications to nucleotides, cationic polymers) may increase stability within the environment and increase uptake of dsRNA applications, thus posing further questions surrounding their environmental fate (Cagliari et al., 2016; Heinemann & Walker, 2019; Rodrigues & Petrick, 2020);

- *Resistance in pests and pathogens*. Due to the sequence specific nature of the technology, it is likely that resistance attributable to changes in sequence can be mitigated through a redesigned molecule. However, questions remain regarding resistance caused by changes uptake of the molecules, as observed in corn rootworm experiments (Khajuria et al., 2018; Wytinck et al., 2020);

- *An understanding of epigenetic changes induced by exogenous RNAi*, especially if they are shown to be hereditable (Dalakouras & Papadopoulou, 2020; Heinemann, 2019); and

- *Differential responses depending on environmental conditions and stage of development*. Off-target testing should include different life stages of organisms, as accumulations of transcripts will be differential during the lifecycle (Vogel et al., 2019).

6.2. Mitigation and management strategies

Among synthetic biologists and in policy discussions, in addition to post-release environmental monitoring, a commonly suggested response to the limitations of physical containment and the possibility of organisms successfully designed for environmental release is that synthetic biology be used to design organisms with "built-in safety features" (SCBD, 2015). As such, following the identification of potential negative impacts on biodiversity associated with the application of synthetic biology to especially bacteria, insects

and plants, a few molecular approaches have been proposed to contribute to risk mitigation strategies. For example, genetic techniques exist that permit the site-specific excision of unnecessary DNA, so that only the sequences of interest remain. Other mechanisms exist, whereby the host organisms contain conditional suicide genes that may be activated under certain conditions. These methods act to prevent the spread and survival of the host organisms in the environment, and to prevent horizontal gene flow to wild or cultivated relatives.

There are a number of general areas of research that aim to develop built-in safety features: site-specific recombination; induced lethality; horizontal and vertical gene transfer prevention; trophic containment; and semantic containment (SCBD, 2015). Some of these, and others, are discussed in the following subsections.

6.2.1. Removing unwanted inserted sequences

Site-specific recombination systems are common in prokaryotes and lower eukaryotes such as yeast, and serve various biological functions (Grindley et al., 2006). The recombinase protein catalyses recombination of DNA between two recognition sites. The outcome of the recombination can be site-specific excision, integration, inversion, or translocation, depending on the position and the relative orientation of the two recognition sites on the DNA molecules (either linear or circular form), and the type of reaction is dependent on enzyme type. The *Cre-lox* site-specific recombination system was first used to remove extraneous genetic sequences in tobacco (Dale & Ow, 1990). Since then, *Cre-lox* or other later-identified site-specific recombination systems (e.g. meganucleases, TALENs and ZFNs) have been used to eliminate undesirable inserted sequences in an ever-widening range of plants (Gidoni et al., 2008; Yau & Stewart, 2013).

6.2.2. Use of virulence factors and synthetic resistance

When considering engineered induced lethality (also referred to as "kill switch" or "suicide gene") (subsection 3.3.3(d)), as discussed by Wright et al. (2013), Schmidt and de Lorenzo (2012), and Moe-Behrens et al. (2013), kill switches in microbes are prone to failure, which has implications for the design of genetically engineered bacterial products for environmental applications (subsection 6.2.3). The selective pressure acting to inactivate or lose suicide genes (e.g. through mutation) is expected to be stronger than for other genes precisely because the suicide genes are expressly designed to kill the host cell. Moreover, while suicide genes are intended to be active only under certain conditions, there may be varying amounts of "leaky" expression, which means that the selective pressure is present even under normal conditions where the host cells are intended to thrive. Wright et al. (2013) corroborate this notion by writing that "dependency devices based solely on toxins seem designed for failure due to their inability to withstand mutation over time".

In a subtle variation of this approach, Kato (2015) reported the construction of an *E. coli* strain that has a synthetic essential gene that is expressed only in the presence of an unnatural amino acid that is an artificial essential nutrient and that therefore cannot survive outside of the laboratory. A modified toxin-antidote system was introduced into the strain, where the antidote is a protein, and an unnatural amino acid translational switch controls the expression of the antidote. The strain can only survive when the antidote protein is produced in the presence of the unnatural amino acid in the laboratory. In the natural environment, the strain should die due to both the absence of the unnatural amino acid and the accumulation of the toxin. Using a similar strategy, where a CRISPR nuclease disrupts an essential gene (toxin), while also carrying a recoded version of that gene (antidote), engineered gene drives of this nature could allow for releases to potentially be confined to a desired geographic location. Using *in silico* predictions, such drives would have a non-zero invasion threshold frequency, meaning that a critical frequency would be required for the drive to spread through the population (Champer et al., 2020). Approaches to use built-in biocontainment strategies in engineered gene drives are discussed next (subsection 6.2.3).

6.2.3. *Genetic biocontainment approaches*

The co-incorporation of a genetically engineered biocontainment system offers an increased ability to help control the spread of modified organisms and mitigate derived risks. In fact, several risk mitigation strategies have been proposed with the most advanced applications being developed based on RIDL, a form of sterile insect technique (Thomas et al., 2000). Another strategy is to develop applications which use engineered gene drives whose non-Mendelian transmission is conditional on the presence of synthetic molecules in the environment of the target species, so that the removal of the synthetic molecule is expected to stop the spread of the gene drive, and natural selection to remove the drive from the population (Amo et al., 2020; Esvelt et al., 2014). However, the development of such molecule-dependent drives is still in its infancy and may have to be tailored for each ecosystem and target species (Rode et al., 2020).

In yet another strategy, specifically for insects, the idea is to introduce resistant individuals carrying a modified target locus that prevents homing ("synthetic resistant" allele; Champer et al., 2016; Vella et al., 2017). However, this strategy results in a modified population with 100% resistant individuals and does not allow the full recovery of the original wild-type population (Rode et al., 2020). In addition, synthetic resistant alleles are predicted to be rather ineffective against replacement drives with small fitness costs (Vella et al., 2017), because of the limited selective advantage of synthetic resistant alleles. Finally, another strategy has been proposed to release suppressor individuals that carry a new piece of DNA which will eventually lead to the knock-out of the initial gene drive (Esvelt et al., 2014; Marshall & Akbari, 2018). These alternative mitigation strategies rely on gene conversion and can be used against virtually any type of CRISPR-based homing gene drive (Rode et al., 2020). Two types can be distinguished:

(1) Those strategies that include the *cas9* gene and that can target either the drive allele only (reversal drives [Esvelt et al., 2014]; overwriting drives [DiCarlo et al., 2015]) or both the drive and wild-type alleles (immunizing reversal drive [Esvelt et al., 2014; Vella et al., 2017]). However, with these strategies, a functional *cas9* gene will remain in the final population, which may increase the risk of subsequent genetic modifications such as translocations, and of possible negative environmental outcomes (Courtier-Orgogozo et al., 2017).

(2) Those strategies that do not encode *cas9* and rely instead on the *cas9* gene present in the initial gene drive construct. They can be contained in a single locus (ERACR: element for reversing the autocatalytic chain reaction, Gantz & Bier, 2015; CATCHA: Cas9-triggered chain ablation, Wu et al., 2016), or be across two loci (CHACR: construct hitchhiking on the autocatalytic chain reaction, Gantz & Bier, 2016). These mitigation strategies may be safer for the environment, due to the absence of a functional *cas9* gene. Thus far, CATCHA brakes, erasing CHACR (e-CHACR; *cas9* inactivation) and ERACR (*cas9* deletion and replacement) have been implemented in the laboratory, demonstrating to be effective at neutralizing an engineered gene drive (Wu et al., 2016; X. R. S. Xu et al., 2020). For the e-CHACR and ERACR systems, the *trans*-acting elements drove to completion within 10 generations with the e-CHACR system copying and replacing *cas9* ~99% of the time (X. R. S. Xu et al., 2020). In progeny assays, CATCHA converted *cas9* between 57% and 85% of the time (Wu et al., 2016). It was predicted that CHACR may be slow to spread due to its two-locus structure, while ERACR may be sensitive to the evolution of resistance at its target sites (*cas9*-flanking sequences whose mutation does not affect enzyme function) (Rode et al., 2020).

However, strategies for remediating effects of gene drive releases suffer from many of the limitations and uncertainties of the engineered gene drives they are designed to undo, e.g. potential for resistance development, efficiency, and off-target effects (Sirinathsinghji, 2019). This can be exemplified by the CATCHA, e-CHACR and ERACR systems, where Sirinathsinghji (2019) suggested

further design considerations are warranted due to unexpected and off-target effects (e.g. the use of two gRNAs, optimizing gRNAs). For CATCHA, Wu et al. (2016) hypothesized that some progeny where the CATCHA did not copy likely contained a non-functional *cas9* due to an insertion or deletion of bases in the genome (an "indel") caused by non-homologous end joining. In the case of e-CHACR, X. R. S. Xu et al. (2020) observed a biased inheritance of donor chromosomes, and attributed this to cutting twice or induced male or homozygous lethality (when located on the X chromosome). For ERACR, the action of the element resulted in damage to the chromosome, in turn resulting in other outcomes, including the failure to delete *cas9*, the deletion of *cas9* without copying the ERACR element, and rare recombination events. Resistance was also observed, but damage caused to the chromosome carried higher fitness costs than retaining the drive element. Thus, X. R. S. Xu et al. (2020) concurred with the recommendation of the US National Academies of Sciences, Engineering, and Medicine (2016b) that the decision to proceed with potential releases of engineered gene drive systems should not be predicated on constructing neutralizing elements and that such systems should be developed only for precautionary purposes.

A more nuanced strategy has recently since been proposed (Amo et al., 2020), called a trans-complementing CRISPR gene drive, which splits the Cas9 (the "toxin") and gRNA (programmed to cut an essential gene on the wild-type chromosome, where cleavage-repair will typically result in a disrupted version of the target gene) into two different transgenic lines. In experiments with *Drosophila*, neither component was able to exhibit gene drive activity when separated, providing the same safety profile as a gRNA-only drive. When combined by genetic cross, however, the two complementary components reconstituted the properties of a full engineered gene drive, resulting in both elements propagating together (Amo et al., 2020). This is because the "antidote" element is a functioning copy of the target gene that is located within the drive allele and is recoded to no longer match the drive's gRNAs so that it is not subject to disruption by the drive (Champer et al., 2020). Thus, individuals that inherit only a toxin-disrupted allele suffer from a toxic effect, while individuals that only inherit the drive, or who inherit both a disrupted allele and the antidote, do not experience the deleterious toxic effect. By this mechanism, the relative frequency of the engineered gene drive should increase over time as wild-type alleles are removed from the population. These findings have implications also for other strategies that similarly use multiple elements driving simultaneously, such as the proposed "daisy-chain drive" (subsection 6.2.4).

6.2.4. Post-release removal

Some synthetic biology developers are beginning to explore the possibility of developing mechanisms by which LMOs containing engineered gene drives could be removed from the environment post-release. Current ideas include gene drives that counter other gene drives (anti-drives), drives with built-in limitations (daisy-chain drives),[75] or underdominance drives (Heffel & Finnigan, 2019). In the case of the anti-drives, a standard reversal system targets only engineered gene drive individuals; immunizing anti-drives are able to target both engineered gene drive and wild-type individuals. For some scenarios, anti-drives systems may not eliminate drives within a population, and, instead, might achieve a stable equilibrium. Moreover, without additional modifications, anti-drives systems require construction at the same locus that the engineered gene drive was originally installed. This might prove challenging in some scenarios where anti-drives are not already engineered and available for release (Heffel & Finnigan, 2019). Daisy-chain

75 A split drive (also known as a daisy drive) is defined as "*A multi-component form of homing drive in which multiple split drives are linked into a chain such that each can exhibit homing and hence biased inheritance (drive) only in the presence of the previous element in the chain. The first element in the chain does not drive; this limits the geographic spread and temporal persistence of the drive components, while allowing more rapid spread ('stronger drive') of the later elements of the chain than would a similar two-component split drive system. Several variants of this system have been described and modelled, including 'daisyfield', with multiple parallel components replacing elements of the linear 'chain' and combinations of daisy drive with underdominance-based systems ('daisy quorum')*" (Supporting information, Alphey et al., 2020).

systems can provide local spread of a drive element but they cannot propagate at the same scale as traditional drives; the goal is limited spread of drives, rather than targeted removal of active drives (Noble et al., 2019). Finally, in the case of underdominance drive systems, proposals for population reversal requires inundation with either wild-type individuals (Champer et al., 2016) or "free suppressor" individuals (Edgington & Alphey, 2018). The incorporation of one of these types of safety mechanisms could potentially provide additional levels of control and programmability that are not currently possible in simple engineered gene drive setups that are designed with only initial parameters and a single outcome. Furthermore, failsafe systems to protect the original wild species, even if never used in application, could be seen as a critical step towards gaining support for the release of LMOs containing engineered gene drives within native ecosystems (Heffel & Finnigan, 2019).

6.2.5. Detection and identification

Approved LMOs are detectable, identifiable, and quantifiable by polymerase chain reaction (PCR) methods, which target the stable integration site of "foreign" DNA elements in a genome (Fraiture et al., 2015). Organisms produced by the application of synthetic biology techniques, for example, engineered gene drives, may be relatively easy to detect and identify; however, those that exploit genome editing, for example, may lack integrations of any foreign DNA or corresponding genetic elements commonly used in genetic engineering. This has important repercussions for the effective detection and identification of synthetic organisms, and especially for those that may fall under existing biosafety regulatory provisions and authorized for international trade (currently plant-based commodities). As explained earlier, the application of genome editing can introduce small changes and aims to minimize the amount of unintended off-target alterations in the target genome. When used in plants, together with subsequent backcrossing and selection steps, the intended alteration may be limited exclusively to the target site without leaving other permanent changes in the genome (Wang et al., 2014). As a result, the genome sequence of a genome-edited plant may differ only minimally from its parental one (Shin et al., 2016), such as the substitution, insertion or deletion (indel) of only a single nucleotide (Grohmann et al., 2019).

For those jurisdictions that predicate their regulations on a process-based approach, if a known insertion is present, PCR-based methods will likely be the method of choice for detection as they are highly specific and sensitive. Based on the experience from LMO testing, it should be technically feasible to establish event-specific PCR methods targeting larger nucleotide sequence changes induced by genome editing (for example SDN-3). Short sequence changes (substitutions or indels of one or a few nucleotides) induced by SDN-1, SDN-2, or ODM should also be detectable using a specific probe, for example, in real-time PCR or digital PCR assays (Stevanato & Biscarini, 2016). Single nucleotide polymorphism (SNP) genotyping approaches can be used to detect very small sequence differences of one or a few nucleotides, provided an adequate reference sequence is available (Huggett et al., 2015). Additionally, off-target effects (e.g. chromosomal rearrangements, satellite mutations, insertions, deletions) could possibly be detected using a combination of (whole genome or targeted) sequencing with bioinformatics analyses. PCR and Southern blot methodologies could also be employed once a specific mutation is known (Lema, 2021).

However, concerns have been raised regarding the feasibility of developing a robust and specific PCR-based quantification assay for the presence of genome-edited material that is applicable for routine testing of composite food samples at levels of 0.9 or 0.1% of genetically modified material (Emons et al., 2018). Despite this, real-time quantitative PCR and droplet digital PCR have recently demonstrated the potential to detect and quantify mutations identical to those resulting from specific genome editing applications in crops (Chhalliyil et al., 2020; Peng et al., 2020). In particular, Chhalliyil et al. (2020) suggested that their method for detecting the single nucleotide variation could be consistent with ISO 17025

standards.[76] However, the European Network of GMO Laboratories noted that the technique lacks the ability to identify the cause of the mutation. Additionally, they noted that further work to improve specificity would be needed, reporting that similar mutations to the one studied by Chhalliyil et al. (2020) also occur in the conserved acetolactate synthase gene in the majority of more than 160 weed species (ENGL, 2020; Nandula et al., 2020).

Thus, despite the ability to detect these specific genomic changes, differentiating the cause of each mutation from a natural occurrence to one derived from a particular technique may not be possible with PCR-type methodologies. To explain, conventional mutagenesis techniques, such as irradiation or mutagenic chemicals, as well as genome editing applications, do not leave specific imprints in the genome, therefore making it impossible to identify the technique applied to change the DNA. After considering the range of molecular options currently available, as well as the extent of data from requisite accompanying documentation e.g. concerning origin and pedigree, Grohmann et al. (2019) concluded that the identification of specific genotypes in heterogeneous samples (commodities) could be expensive, time-consuming, and technically challenging (potentially impossible) due to the likely reliance on whole genome sequencing and complexity of certain plant genomes. Further, the authors noted that validation of an event-specific detection method and its implementation for market control may only be feasible for genome-edited plant products carrying a known DNA alteration that has been shown to be unique. Thus, to assist with some of these complexities, it was suggested that there could be a need for an anticipatory framework to exchange data and exercise (voluntary) information disclosure practices to establish sufficient information for identifying specific genome-edited products, if such organisms were regulated in their source country or country of import (Ribarits et al., 2021). Such an approach would be useful for all legislation imposing regulatory requirements for genome-edited plants and thus would strongly benefit from a coordinated international collaboration, e.g. under the umbrella of the Convention. Others, however, consider the technological problems to be surmountable should there be the political will (Kawall et al., 2020).

For organisms produced through other types of synthetic biology tools, DNA watermarks or barcodes, i.e. unique synthetic DNA sequences embedded in multiple loci of synthetic genomes, were originally proposed for isolating or identifying and tracking synthetic organisms, especially microbes (Jupiter et al., 2010). This idea has since morphed into "DNA signatures", but through the advancement of technologies, inherent vulnerabilities have been identified which may intentionally be exploited to support the counterfeiting of the synthetic host organism necessitating the potential re-conceptualization of how DNA signatures may reliably contribute to the identification and traceability of synthetic organisms (Mueller, 2019).

Apart from considerations related to DNA, proteins could also facilitate detection and identification of organisms produced through synthetic biology. It is likely that a (novel) protein expressed by an organism would allow for protein-based detection methodologies (Alarcon et al., 2019). Further, it was proposed that minor changes can deliberately be made to the protein sequence of the synthetically produced protein as a positive identification tool ("label") (CITES 2018). Further, organisms produced through synthetic biology could have an additional fluorescent protein marker, such as the DsRed2 protein in the case of Oxitec's self-limiting insects (subsection 3.2.2(a)), for visual detection via a suitable epifluorescent microscope (Beech et al., 2012; Romeis et al., 2020). Overall, for organisms produced through synthetic biology, it was highlighted that although experience and technical capacities were currently lacking, technologies continue to be developed and could be tested for feasibility (Keiper & Atanassova, 2020).

Regarding the products of synthetic biology, current methods for the detection and identification

76 ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories; https://www.iso.org/standard/66912.html.

of LMOs may be applicable, depending on the circumstance. For example, certain modifications could be made to products to facilitate their detection and identification. For example, it has been suggested that a DNA watermark containing a DNA sequence not naturally present in natural horns could be incorporated into the synthetic product, such that DNA detection techniques (e.g. PCR) could be applied (Bonaci & Markus, 2019). Another example could relate to the nucleic acid-based techniques for dsRNA molecules. Often, these molecules can be detected using Northern blots,[77] but to avoid the labour-intensive nature of Northern blotting, PCR-based and bead-based techniques may have also been proposed (Bachman et al., 2020; Fischer et al., 2016; Kaldis et al., 2018; K. Zhang et al., 2020). RNA sequencing, as applied in microbiology and plant virology, could also be broadly applicable for identifying dsRNA and siRNA molecules, but research has yet to be done to demonstrate their applicability (Hadidi et al., 2016; Schroeder et al., 2016). Further, the detection and identification may not be possible for processed products (e.g. cooking oil and white sugar), where dsRNA molecules could be removed or degraded (McLoughlin et al., 2018; Rodrigues & Petrick, 2020). For products composed of proteins, immunological methods could be employed for their detection (Sasikumar et al., 2016). However, other techniques not commonly utilized in LMO detection could also be applied, such as mass spectrometry to characterize the protein composition of a biological product (Rathore et al., 2018) or microscopic analyses to visually assist with the identification of a biomanufactured product (e.g. bone) (Bhattarai et al., 2018).

For other products, such fine chemicals or synthetic replacement of natural products, which may not contain DNA or protein, analytical chemical techniques may be required. Drawing on experience from the natural products industry and food forensics, trace or contaminating substances may assist in the differentiation between synthetic and natural products (Girme et al., 2020; Primrose et al., 2010). For example, high performance liquid chromatography and nuclear magnetic resonance spectrometry have been successful at differentiating between synthetic and natural turmeric extracts (Girme et al., 2020; Kim et al., 2021). Another technique that may also be applicable is stable isotope analysis, which details the radioisotope ratios of chemical elements, such as carbon, oxygen, hydrogen and nitrogen, to suggest the geographical region or feedstock source (Primrose et al., 2010; van Leeuwen et al., 2018). Despite the increased availability, it is important to recognize that further research is required to test the applicability and feasibility of these techniques for the detection and identification of synthetic biology products.

77 Northern blotting is an RNA analysis technique that relies on the separation of RNA molecules according to size using an electric field (electrophoresis), followed by transferring and cross linking to a nylon membrane, and visualization using a labelled probe complementary to the sequence of interest (He & Green, 2013).

D. Synthetic Biology Governance and Regulatory Perspectives

This part of the document first discusses the various regulatory approaches that are emerging following the authorization and commercialization of the first applications of synthetic biology (section 7), before focusing more extensively on international conversations and structures (sections 8 and 9).

7. The governance and regulation of synthetic biology

Now that products of synthetic biology are entering advanced stages of development and are beginning to become commercially available (section 3), this is bringing challenges to building consensus on whether (in some cases) and how they are to be regulated, either under the same regimes as genetic engineering, albeit with adaptations, or under new regimes yet to emerge (Lema, 2021). The current debates echo a similar range of views expressed at the emergence of genetic engineering (Keiper & Atanassova, 2020): from biotechnological developments being inherently risky and requiring stringent regulation based on the precautionary approach, through to these technologies not presenting any unique or novel risks. If discussions to date are anything to go by, those likely to fall under regulation will be subject to a thorough analysis of their different potential impacts, both directly (section 4), and more broadly (section 5) on biodiversity-related issues and others before any authorization will be given. Again, although not attempting to provide comprehensive coverage of all governance structures and approaches, examples are provided of some of the various regulatory practices that have emerged concerning synthetic biology and the discussions around these.

7.1. Current regulatory practices and approaches related to synthetic biology

As mentioned in the previous section, requests for use and release of synthetic biology applications will likely be evaluated within a risk-based regulatory decision-making process which will be influenced by a range of broader considerations, including ethical, socio-cultural, epidemiological, ecological and economic aspects. Furthermore, potential risks of synthetic biology must be weighed against the potential benefits and considering that there could also be ethical components to the decision to use or not a new technology.

As regulatory clarity is increasingly being sought as more and more synthetic biology applications proceed through advance development to commercial activity, regulatory authorities have begun to publish the results of discussions that they have had in order to better inform developers, decision makers and the wider public of how they will interpret their legal framework in this light. The main technologies that have received increased regulatory scrutiny to date are genome editing, engineered gene drives and RNAi technology, and these are discussed below.

7.1.1. Genome editing

Different positions are taken by regulatory authorities in countries across the globe when addressing whether applications using genome editing fall within their regulatory purview; positions that largely depend in most cases on whether modifications are recognized as comparable to those arising via spontaneous processes or introduced with the use of conventional mutagenesis tools such as irradiation or chemical treatment, or comparable to modifications achieved using transgenic approaches (Custers et al., 2019; Menz et al., 2020). For instance, those genome editing applications that do not aim at the insertion of foreign (or exogenous) DNA, but at inducing site-specific mutations at single loci of

a plant's own genetic material, are able to create organisms identical to those that could have theoretically come into existence naturally or through conventional breeding. Thus, although a few regulators in some countries have instituted mechanisms for addressing the regulatory status of crops derived from genome editing (Whelan & Lema, 2015; Wolt et al., 2016), decisions as to whether or not they require legal regulation lag behind in many countries (Duensing et al., 2018).

At one end of the range of regulatory approaches is the creation of exclusions by a number of governments for certain categories of genome editing technologies or products where these could have also been obtained through spontaneous processes or through the use of other (conventional) tools and methods (Dederer & Hamburger, 2019). Some of these countries have implemented such exclusions based on their implementation of the definition of "modern biotechnology" characterized by the Cartagena Protocol on Biosafety, whereby a "*novel combination of genetic material*" does not involve DNA changes that could have been obtained spontaneously or with the use of other methods. In these cases, the organism is managed in the same way as other non-LMO organisms (Keiper & Atanassova, 2020). Others apply specific conditions such as undertaking a public consultation, publishing those decisions or introducing the exemptions in specific registers, requiring specific follow-up or monitoring reports. In an example, in December 2020, Japan's Ministry of Health, Labour, and Welfare and the Ministry of Agriculture, Forestry, and Fisheries decided that a CRISPR-Cas9 edited tomato containing elevated levels of gamma-aminobutyric acid would not be regulated as an LMO, thus not requiring a safety assessment associated with LMOs (United States Department of Agriculture, 2020). In the USA, a non-Party to the Cartagena Protocol on Biosafety, a similar regulatory position was taken for a range of applications developed using TALEN by Calyxt, Inc., including a potato with improved processing characteristics, a high oleic soya bean, a high oleic/low linoleic soya bean, a cold-storable potato and a powdery mildew resistant wheat (Calyxt, 2017). Further, the US Department of Agriculture announced in 2018 that it was exempting "*plants generated using plant breeding technologies that have non-templated insertions and deletions and that have a single base pair substitution, because they could otherwise be created by conventional breeding and pose no increased plant pest risk relative to their conventionally bred counterparts*".[78] Similarly, Argentina, Australia, Brazil, Chile, Colombia, Japan, Nigeria, Paraguay, the Russian Federation and Israel (some of which are non-Parties to the Protocol) have established policies and/or guidance describing which genome-edited applications are not required to follow LMO regulation (in this case), and with particular reference to those where the final products do not contain foreign DNA sequences (Entine et al., 2021; Ku & Ha, 2020; Lema, 2019; Obukosia et al., 2020). In a slightly different approach, in Canada, plants, animals and their derived products (food, feed) produced through genome editing are regulated and subject to assessment based on whether any novel traits are expressed (Ellens et al., 2019).

At the other end of the range is the position taken by both Europe and New Zealand, for instance, which have upheld the legal ruling that genome-edited applications should be regulated in the same way as LM crops (Fritsche et al., 2018; Park et al., 2019). In contrast to the above position of the US Department of Agriculture in relation to plants, the US Food and Drug Administration (FDA) has proposed mandatory pre-market evaluations for all food animals whose genomes have been intentionally altered using modern molecular technologies, including genome editing technologies (Van Eenennaam, 2019). This will require companies to seek a separate approval for the same genomic alterations in each new lineage into which it is introduced. Thus, animals with an altered genome and from the same lineage would be considered to be containing an animal drug, including those that acquire the alteration through cross-breeding (FDA, 2021). Continuing with animal applications, Argentina updated its regulations on animal biotechnology in 2017 to

78 https://www.aphis.usda.gov/brs/fedregister/BRS_2020518.pdf.

include new technologies such as genome editing, and so genome-edited animals will also be subjected to risk assessment in contrast to the above position with plants if no foreign DNA sequences are present. In China, discussions regarding regulation and risk assessment began in 2015 (Whelan et al., 2020). A working group within the National Biosafety Committee was established in 2016 to provide technical assistance on the risk assessment of new breeding techniques, including genome editing (Gao et al., 2018). As an interim policy, China will regulate genome-edited agricultural products under LMO legislation. As of 2020, no formal regulations had been issued regarding genome editing (D. Zhang et al., 2020).

Developing countries, especially many in Africa, South and South-East Asia, are gradually reviewing their regulatory frameworks in order to address genome editing, and a preliminary appraisal indicates that many are most likely to use a science-based approach in developing regulatory frameworks to ensure that their regulatory decision-making is predictable, consistent and efficient (Obukosia et al., 2020). For example, in 2020, the Indian Department of Biotechnology published draft guidelines for genome editing regulation that require additional safety and efficacy testing for genome-edited crops.[79] In another example, Biosafety South Africa, an organization under the Department of Science and Technology that provides science-based advice to the regulatory authorities, concluded in 2019 that while genome-edited organisms are not necessarily LMOs, they will still have to comply with relevant legislation to ensure their sustainability (Biosafety South Africa, 2019).

7.1.2. Engineered gene drives

Although none of the applications using engineered gene drives are ready for release yet, they attract much attention in the scientific literature and from the media and regulators. This is mainly because the release of self-sustaining LMOs into the environment – deliberate or not – potentially has the ability to elicit long-term, large-scale and 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.

As these types of applications may spread across jurisdictional boundaries following authorized release, it has been suggested that regional approaches to facilitate international regulatory oversight and approval could better serve their governance (Devos et al., 2020). Likewise, for engineered gene drives, spread and persistence are their *raison d'ĕtre*, posing different legal and regulatory challenges because of their high potential to spread beyond national borders (Ching & Lin, 2019).

The regulation of LMOs containing engineered gene drives has been a polarized issue that has raised concerns on different areas, such as the application of the precautionary principle and the obtention of FPIC of IPLCs (Dolezel et al., 2020). These issues are explored further in subsections 5.1.2 and 9.3.2.

Also, while some groups favour continued laboratory research and development of LMOs containing an engineered gene drive in order to elaborate a greater understanding of the technology and advance potential benefits, others draw attention to alleged gaps in regulatory oversight and the potential for serious ecological and societal consequences to reinforce calls for a moratorium on their environmental release (Civil Society Working Group on Gene Drives, 2016; Dolezel et al., 2020).

So far, LMOs containing engineered gene drives fall under the definition of LMO as per the Cartagena Protocol on Biosafety (SCBD, 2020e), and therefore the provisions of the Protocol will apply to these organisms. In addition, as will be described below in section 8.1, these organisms will also be covered under the Convention on Biological Diversity based on Articles 8(g) and 19(4). Some stakeholders are of the view that the Convention on Biological Diversity and its Protocols, where there have been discussions

79 https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/india-crops-food/.

and decisions on the issues of LMOs containing engineered gene drives, are currently the best home for their international governance (Ching & Lin, 2019).

7.1.3. RNA-based technology

Genetically modified RNAi plants are being assessed and regulated using existing LMO regulatory frameworks. However, there may be a need to adapt existing plant protection product legislation so that it incorporates appropriate science-based risk assessment procedures for topical RNA-based applications (Heinemann, 2019), if it doesn't already do so. This is reflected in the current activities of the OECD working group on pesticides (Mendelsohn et al., 2020; Mezzetti et al., 2020).

Regarding the use and regulation of exogenous RNA-based applications, some researchers expect that they will unlikely be regulated in a similar manner to LMOs due to the non-transgenic nature of the product (Cagliari et al., 2019). This conclusion was also reached by Darsan Singh et al. (2019), who examined the regulation of RNAi-based technology in India and Malaysia. They noted that discussions regarding exogenously applied dsRNA pesticides had yet to begin but believed that synthetic RNA would not fall under the definition of LMO in either country, as the molecules could not be considered living organisms. Rather, the authors suggested that dsRNA applications may be regulated under different legislation, such as the Indian Insecticides Act 1968 or the Malaysian Pesticides Act 1974. However, they also found that plants or other organisms containing RNAi-based constructs would be considered transgenic in line with LMO legislation in both countries. There are, however, differing views on this point, principally against the contention that topically applied dsRNA would not result in any heritable changes (Heinemann, 2019; Heinemann & Walker, 2019).

Thus far, two countries have taken specific decisions on exogenously applied RNA-based applications. The New Zealand Environmental Protection Authority issued a decision that makes the use of externally applied dsRNA molecules on eukaryotic cells or organisms technically out of the scope of legislation on new organisms, making risk assessments of such treatments in the open environment unnecessary[80] (Heinemann, 2019). In Australia, topically applied RNA-based products are not regulated as GMOs for the purposes of the *Gene Technology Act 2000*. A new provision issued on 8 October 2019 (within the *Technical Review of the Gene Technology Regulations 2001*) clarified that "*techniques involving the application of RNA to an organism to temporarily induce RNAi do not constitute gene technology, provided that the RNA cannot be translated into a polypeptide, the organism's genomic sequence cannot be altered as a result, and an infectious agent cannot be produced*". Thus, these products will be regulated as a chemical under the *Agricultural and Veterinary Chemicals Code Act 1994*. Data packages in support of the registration of novel agricultural chemical products address, at a minimum, chemistry and manufacture, human health, worker health and safety, environmental fate and toxicity, efficacy and crop safety, and overseas trade (Fletcher et al., 2020).

7.2. The scope of national regulatory frameworks and their wider implications

With the exception of a few regional approaches, the majority of regulatory decision-making regarding the authorization of synthetic biology applications is expected to be made at the national level. Still, national decisions are taken within a broader context. Firstly, even at a national level a decision may be directed by different policies addressing environmental protection, health and welfare, science and technology. Secondly, each of these policy areas is likely further connected to international policies and agreements, possibly with overlapping mandates (see section 9.2). For example, international law has an important bearing on the authorization and eventual trade in biotechnological products, the most familiar example being the trade in genetically modified organisms or products derived from them. Some regulations may be relevant to proposed applications of synthetic biology, such as the global moratorium on ocean fertilization (for ameliorating climate change

80 https://www.epa.govt.nz/assets/FileAPI/hsno-ar/APP203395/APP203395_Decision.-Superceded-June-2021.pdf.

by promoting oceanic carbon dioxide uptake) under the Convention on Biological Diversity and the provisions of the Biological Weapons Convention (BWC; see subsection 9.3.1(b) for further reading). The Environmental Modification Convention (ENMOD), an international treaty prohibiting the military or other hostile use of environmental modification techniques such as alterations to weather patterns or ocean circulation, may also apply to some possible uses of synthetic biology. The reader is directed to later sections (e.g. subsection 9.3.1(b)) for further discussions in this area.

A heterologous regulatory arena coupled with uncertainty in the regulatory environment could discourage private and public sector investment into the development of applications for the public benefit (Komen et al., 2020). Using experiences with conventional biotechnology as a proxy, hurdles faced by countries with emerging national regulatory frameworks typically include lack of inter-ministerial collaboration and harmonization, post-release requirements and high-level political will wavers (Komen et al., 2020).

In recognition of past experiences with other emerging technologies, some countries are beginning to be proactive in setting an enabling policy landscape concerning synthetic biology. The UK Synthetic Biology Strategic Plan 2016 (Synthetic Biology Leadership Council, 2016) is an example of a national focus on the responsible acceleration of commercial delivery of new products and services of public benefit and which emphasizes the need for responsible research and innovation, and proportionate and adaptive regulation for the maximization of public benefit and minimization of risk. It also suggests the development of technical standards at the national level to support the acceleration of commercialization (The British Standards Institution, 2015). These standards could also assist regulators and contribute to international discussions on appropriate regulatory and governance systems for synthetic biology. For example, UK Research and Innovation, a public funding body of the Department for Business, Energy and Industrial Strategy, imposes responsible research and innovation requirements on funding recipients,[81] supported by guidance, workshops and outreach activities including publishing a Responsible Innovation Guide in conjunction with the British Standards Institution.[82]

A series of reports from the US National Academies of Sciences, Engineering, and Medicine (NASEM) addressed applications, products and enabling technologies that are included in the scope of synthetic biology. In their 2017 report on the "future products of biotechnology", NASEM reached the conclusion that the "*…scale, scope, complexity, and tempo of biotechnology products are likely to increase in the next 5–10 years. Many products will be similar to existing biotechnology products, but they may be created through new processes, and some products may be wholly unlike products that exist today*" (National Academies of Sciences, Engineering, and Medicine, 2017). NASEM emphasized the need for regulatory systems to have the agility to rapidly adapt to technological change and manage the assessment of a greater diversity of products (National Academies of Sciences, Engineering, and Medicine, 2017).

Australia's Gene Technology Ethics and Community Consultative Committee has stated that, as of 2012, known proposed applications of synthetic biology do not raise new ethical issues and would be regulated under the existing Australian legislation.[83] In 2018, a key outcome of a horizon scanning process by the Australian scientific community called for their already progressive and effective regulatory framework to remain so, by responding to technological developments in a timely manner and ensuring regulation that is proportionate to risk (Gray et al., 2018).

Mirroring an earlier Opinion of the European Commission and the Scientific Committees on Consumer Safety (SCCS), on Health and Environmental Risks

81 https://www.ukri.org/about-us/policies-standards-and-data/good-research-resource-hub/responsible-innovation/.
82 https://pages.bsigroup.com/l/35972/2020-03-17/2cgcnc1.
83 https://webarchive.nla.gov.au/awa/20170218212752/http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/gteccc-comm-May2012-htm.

(SCHER) and on Emerging and Newly Identified Health Risks (SCENIHR) in 2014,[84] the German Central Committee on Biological Safety in 2018 also concluded that most synthetic biology approaches result in LMOs that can be assessed according to the existing German regulatory framework, the applicable European Directives (2001/18/EC and 2009/41/EC), and the Cartagena Protocol on Biosafety. Specifically, their assessment concluded that the insertion of synthetic genes, gene circuits, metabolic pathways, or entire genomes in an organism results in an LMO as defined by these regulatory frameworks. They also concluded that the reduction of a genome to create a minimal cell, and the use of xenonucleic acids to create bioorthogonal systems are approaches that result in LMOs within the scope of existing regulatory frameworks. Further, they concluded that these developments did not present specific risks in addition to those already assessed for LMOs developed using recombinant DNA technologies (Zentrale Kommission für die Biologische Sicherheit, 2018).

The South African regulatory system considered that it already has a well-established GMO regulatory system based on a holistic approach that considers both biosafety aspects and socioeconomic considerations in decision-making and which provides a robust framework to regulate activities with any synthetic organism considered to be an LMO as well as their products. It was concluded that discussions on synthetic biology are therefore considered in the context of biotechnology and in the legislative framework of biotechnology and LMOs (Rhodes & Mandivenyi, 2020).

Similarly, in 2019, the National Biosafety Management Agency of Nigeria amended the *National Biosafety Management Agency Act 2015* to account for new developments in synthetic biology. With the amendment, the scope of the act was enlarged to cover emerging issues in modern biotechnology. Thus, a person, institution or body would need approval of the Agency before working with engineered gene drives, genome editing and synthetic biology (National Biosafety Management Agency, 2019).

7.3. Self-regulation by the scientific community and moratoria

In this section, illustrative examples are provided of self-regulation by the scientific community, commencing with the Asilomar declaration of 1975 and subsequent calls for moratoria relevant to synthetic biology. Self-regulation is also considered in the context of the annual iGEM competition which since 2003 gives students the opportunity to push the boundaries of synthetic biology by tackling everyday issues facing the world. Such initiatives can and do influence discussions at the international level and therefore can also have potential implications on the governance of synthetic biology.

Self-regulation in this context does *not* mean that scientific practices are unregulated by national or other levels of government. Rather, it refers to a portion of the scientific community agreeing among themselves on certain conduct, generally additional to any existing legal or regulatory obligations. Self-regulation is sometimes discussed as an option *in lieu of* formal statutory oversight (see Balmer & Martin, 2008), but it is rarely a matter of either/or.

7.3.1. Asilomar declaration

In the past, scientists in biotechnology have practiced self-regulation. In 1975, scientists in the USA working on recombinant DNA technologies agreed to a short-lived moratorium on some aspects of their work through a declaration (Berg et al., 1975) issued in 1975 at the Asilomar Conference on Recombinant DNA Molecules, which was attended by 140 scientists, predominantly from public institutions around the world, as well as lawyers, government officials and members of the media (Keiper & Atanassova, 2020). The moratorium involved deferring experiments on highly pathogenic organisms, genes coding for toxins, and large-scale experiments, and containment safeguards for continuing research.

After Asilomar, precautions for recombinant DNA (rDNA) experiments gradually relaxed thereby laying the foundations for many of the technologies which underpin synthetic biology today. This relaxation has been attributed to the low incidence of

84 https://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_21_en.

accidents (Schmidt and Lorenzo 2010) and a "culture of safety" (Erickson et al., 2011) involving rDNA despite its increased use. Critics of self-regulation see the Asilomar declaration as a strategic move to pre-empt greater government oversight and narrow the focus of concern (ETC Group, 2007).

The acknowledgment at Asilomar of uncertainties around hazards of rDNA and the difficulty in obtaining accurate estimates of risk is heralded as the beginning of precautionary biosafety regulation in this field (Berg et al., 1975; Berg & Singer, 1995; Keiper & Atanassova, 2020). As emerging technologies in this field continue to evolve, concerns about safety and appropriate regulatory oversight that brought about the Asilomar Conference persist. In the decades since Asilomar, the focus of such debates has moved away from scientific conferences and into the fora and processes of the Convention associated with biosafety and risk assessment. Some have welcomed this transition, arguing that Asilomar-like self-governance is an inappropriate model for emerging technologies such as synthetic biology. Bennett et al. (2009) argue against assumptions of a cohesive community of experts that can exclude the public and make "gentlemen's agreements" in today's context of aggressive patenting, internet news, and global security conditions. Others lament that such Party (or government)-led processes in which the scientific community can only "observe" unless they are directly engaged by governments, has resulted in debates and discussions that are relatively lacking in participation by its practitioners and they advocate for more active involvement by the scientific community in order to drive efficient, science-based regulation (Keiper & Atanassova, 2020).

7.3.2. Post-Asilomar calls for moratoria

Echoes of Asilomar were apparent in the *de facto* moratorium on genetic use restriction technologies (GURTs) agreed in 2000 at the fifth meeting of the Conference of the Parties to the Convention. They are also apparent in the moratorium agreed to halt ocean fertilization activities other than legitimate scientific research to be assessed on a case-by-case basis, until scientists better understand the potential risks and benefits of manipulating the oceanic food chain, adopted in 2008 under the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (London Convention) and subsequently reinforced by decisions under the Convention on Biological Diversity addressing biodiversity and climate-related geoengineering (decision IX/16 C in 2008 and X/33 in 2010).

Synthetic biologists have talked about self-regulation and have made some concrete progress. The 2006 "SB2.0" international conference on synthetic biology was initially anticipated to produce an "Asilomar-like" declaration, particularly with regard to the need for screening sequences. There are differing accounts as to why the draft declaration was never voted on or passed. According to some, there was concern that a call for self-regulation would be seen as "closed-shop" governance, and that society generally is "different" now (Campos, 2009; Service, 2006). The ETC Group (2007), on the other hand, suggested that there was internal disagreement over whether or not to boycott non-compliant gene synthesis companies. Despite the disagreement on the broader issue of synthetic biology, there have been efforts to develop, for example, approaches to safeguard gene drive experiments in the laboratory (Akbari et al., 2015), principles for gene drive research (Emerson et al., 2017), and a code of ethics for gene drive research (Annas et al., 2021).

Discussions in recent years concerning self-regulation have focused on the environmental release of LMOs containing engineered gene drives, with negotiators at the fourteenth meeting of the Conference of the Parties petitioned by over 200 mainly civil society organizations to consider contentious language that called upon signatories to "*refrain from the release, including experimental release, of LMOs containing engineered gene drives*".[85] Although falling short of a moratorium, decision 14/19 calls for caution regarding the release of engineered gene drives. Some researchers in the scientific community also

85 As per undated letter '*A Call to Protect Food Systems from Genetic Extinction Technology: The Global Food and Agriculture Movement Says NO to Release of Gene Drives*', accessible at https://www.etcgroup.org/sites/www.etcgroup.org/files/files/forcing_the_farm_sign_on_letter_english_web.pdf.

caution that regulatory gaps must be filled before engineered gene drives can be used in the wild and call for integrated risk management of environmental and security risks, which include, *inter alia*:

- Long-term studies to evaluate the effects of engineered gene drive use on genetic diversity in target populations; and

- Multidisciplinary teams of experts to develop scenarios on deliberate misuse environmental and security risks (Oye et al., 2014).

Conversely, Keiper and Atanassova (2020) caution about the conflation of scientific assessment concerning biosafety with broader political and societal issues in favour of a more evidence-based approach, and for discussions under the Convention on Biological Diversity to better acknowledge the demonstrated, or supporting the potential contribution, of biotechnology toward the achievement of the biodiversity and sustainability objectives at the heart of the Convention. They call on stronger involvement by the scientific community in the discussions under the Convention as essential to support evidence-based decision-making and the development and/or adjustment of effective, adaptive and proportionate regulation (Keiper & Atanassova, 2020). While there is therefore no consensus among the scientific community themselves on the most appropriate approach to self-regulation or administration, it may be necessary to incorporate sufficient safeguards to ensure transparency and accountability to society at large (Akbari et al., 2015; Long et al., 2021), such as the publication of core commitments for field trials of engineered gene drives by a group of developers, ecologists, conservation biologists, and experts in social science, ethics, and policy (Long et al., 2021). These approaches of course are no substitute to appropriate international regulation and governance of synthetic biology application.

7.3.3. International Gene Synthesis Consortium (IGSC)

In 2009, several of the largest DNA synthesis companies came together to form the International Gene Synthesis Consortium (IGSC), a trade industry organization with the objective of promoting the beneficial application of gene synthesis technology while safeguarding biosecurity. Representing approximately 80% of commercial gene synthesis capacity worldwide, IGSC members apply a common protocol to screen both the sequences of synthetic gene orders and the customers who place them. IGSC collaborates with national and international government organizations and other interested parties to curate a Regulated Pathogen Database derived from international pathogen and toxin sequence databases (International Gene Synthesis Consortium, 2017). Other industry bodies such as the Biotechnology Industry Organization support commercial surveillance which is voluntarily undertaken and overseen by industry. They argue that commercial self-regulation in DNA synthesis is sufficient, because "*(at) this early stage of development, synthetic biology does not pose novel threats that are fundamentally different from those faced by the current biotechnology industry*" (Erickson et al., 2011). It has been suggested that such voluntary screening can be improved through "know your customer" vetting standards which are common in finance and adopting "red teaming" attack-simulation approaches which are common in cybersecurity (Diggans & Leproust, 2019). It has also been suggested that such screening should be applied more broadly across the synthetic biology supply chain in order to minimize risk and maximize safety. For example, by lowering the cost of screening and making open-source annotation resources and tools available, a much wider array of synthesis companies will be able to screen their orders (Diggans & Leproust, 2019). In the report "Biodefence in the Age of Synthetic Biology", the US National Academies of Sciences, Engineering, and Medicine (2018) observed that synthetic biology is being pursued overwhelmingly for beneficial purposes, ranging from reducing the burden of disease to improving agricultural yields to remediating pollution; however, it also noted that it can also be deployed maliciously. It acknowledged that although norms of self-governance are not going to deter or prevent a determined malicious actor from seeking to develop, obtain, or use a biological weapon (whether it is enabled by synthetic biology or not), such norms provide groundwork that could be built

upon and at a minimum, they offer a basis for social surveillance of unethical or malicious behaviour within the scientific community.

7.3.4. Do-it-yourself biology ("DIY Bio")

Do-it-yourself biology (DIY Bio) is a growing biotechnological social movement in which individuals, communities, and small organizations study biology and life sciences using methods typical of those of research institutions.[86] Activities may be carried out as a hobby, as a not-for-profit endeavour for community learning and open-science innovation, or for profit, to start a business. One such example is the international synthetic biology competition known as iGEM (section 1.10), which is also an example of a self-regulating community. In the latter regard, iGEM implements a dedicated Biosafety and Biosecurity Program with an adaptive risk management approach which covers activities throughout the competition life cycle, from project design to future application. The Program addresses both traditional (pathogen-based) and emerging risks both in terms of new technologies and new risks with clearly described roles and responsibilities for all members of the community. It makes use of specific tools to gather and review biosafety and biosecurity information, making it easier for those planning and conducting science and engineering to recognize potential risks and match them with appropriate risk management approaches, as well as for specialists to review this information to identify gaps and strengthen plans (Millett et al., 2019). The Program is overseen by the Safety and Security Committee,[87] which consists of experts selected from governments, industry, and academia, to advise on potential safety and security issues for the projects entered into the competition. A white list of approved organisms and parts is published for every competition to guide participants in understanding which organisms and parts require approval before use (Millett et al., 2019). Organisms and products not on the list require approval before use and a partner organization screens the Parts Registry for potentially hazardous parts on a regular basis having regard to the origin, function and risk of the parts (Millett et al., 2019). Further, iGEM's safety policies stipulate that all projects are constrained to laboratory settings (i.e. not for open release into the environment) and devoid of activities deemed risky (e.g. experiments involving engineered gene drives, human experimentation, antimicrobial resistance and biosafety level 3 and 4 organisms).[88]

The iGEM competition describes itself as a "*unique sandbox for testing and improving risk management and mitigation practices*" and collaborates with the broader scientific research community to disseminate lessons learned (iGEM, 2021). For example, in 2019 this included a workshop with the North Atlantic Treaty Organization ("Security and Resilience for Emerging Synthetic Biology and Biotechnology Threats", July 2019), a workshop with the Centre for the Study of Existential Risk ("Novel Practices of Biosecurity Governance", July 2019) and a working meeting with the Nuclear Threat Initiative ("Biosecurity Innovation and Risk Reduction", April 2019).[89]

Likewise, another example of self-regulation in the area of "DIY Bio" can be found in the Community Biology Biosafety Handbook,[90] an open manual that offers biosafety protocols, practices and recommendations aimed specifically at community biology initiatives. Authored by biosafety experts and community laboratory leaders, the manual includes biological, chemical, and equipment safety, as well as specific citizen science topics such as interview practices for screening potential lab members. Given that biotechnology, synthetic biology and community biology are rapidly evolving, the manual was conceived as a living document, to be edited, updated and expanded by the community members.

86 https://en.wikipedia.org/wiki/Do-it-yourself_biology.
87 https://2020.igem.org/Safety/Committee.
88 As per their Safety Rules: https://2020.igem.org/Safety/Rules.
89 https://igem.org/Safety.
90 https://www.genspace.org/community-biology-biosafety-handbook.

7.4. Intellectual property considerations related to synthetic biology and biodiversity

Intellectual property (IP) rights for synthetic biology have been described as a potential "perfect storm"; biotechnology and software already pose serious challenges to the patent system, and synthetic biology's combination of those two areas presents significant challenges (Rai & Boyle, 2007). Concerns persist which echo concerns voiced in the biotechnology sector more broadly that overzealous IP protection will lead to overly broad and ambiguous patent claims or patents over platform technologies which restrict the innovation of others (Henkel & Maurer, 2007; Oye & Wellhausen, 2009; Torrance, 2010). Narrow patents, on the other hand, can cause patent "thickets", where complex designs that incorporate many individual parts face an unmanageable number of patents (Henkel & Maurer, 2007; Rai & Boyle, 2007; Rutz, 2009). Concerns voiced by civil society and public sector organizations have been raised regarding who will and who will not benefit from the applications of synthetic biology, particularly in the agricultural sector in which corporate consolidation, patent proliferation and food security potentially combine in a new "perfect storm" (Pixley et al., 2019). Whether and to what extent IP protection constrains rather than enhances innovation merits further academic analysis; however, the challenges faced in reaching the market by those products that directly target poor people may more closely reflect the regulatory hurdles and investment required in getting a technology to market rather than issues with IP protection and licensing on their own (Divanbeigi & Saliola, 2017). There is also the possibility that, like with electronics and software, a tipping dynamic will lead to one solution dominating an industry because it is the first to establish common standards (Henkel & Maurer, 2007, 2009). Only time will tell; however, the experience with CRISPR-Cas technologies over the past decade looks promising as, despite a high-profile patent dispute, widespread licensing of critical patents associated with the technology has fuelled an explosion of research from both academic and commercial sectors which is transforming life-sciences research (Sherkow, 2018), including synthetic biology applications which are approaching commercial release,[91] for example, engineered gene drives (see sections 1.5, 3.1 and 3.2).

As the field of synthetic biology develops, two main models of IP management for synthetic biology components, organisms, products, and techniques have emerged (Calvert, 2012; van den Belt, 2013). The first is the more traditional approach that heavily relies on patent protection as a means of incentivizing investment in R&D and is based on "*the presumption that unprotected free knowledge will deliver sub-par or even null financial returns to its creators, which in turn would lead to under-investment in research and innovation, under-productive markets, and poorer economic and social outcomes*" (Ribeiro & Shapiro, 2020). This is exemplified by scientists at the J. Craig Venter Institute who applied for a "minimal bacterial genome" patent (Calvert, 2012; Glass et al., 2007). Although ultimately abandoned, NGOs and commentators expressed concern at the breadth of its sweeping claims (Calvert, 2012; ETC Group, 2007, 2011) particularly in relation to the creation of synthetic organisms for the production of biofuels like ethanol and hydrogen (van den Belt, 2013).

The other main model is one that has more recently emerged, the BioBrick system, which is modelled on open-source software. On the iGEM's Registry of Standard Biological Parts, contributing researchers post their BioBrick parts (DNA sequences that incorporate standardized sections) on pages accessible to the general public, which allows users to exchange parts and share their experience. Following a similar philosophy of exchange, the BioBricks Foundation has independently developed a BioBrick Public Agreement that is essentially a contractual agreement between "Users" and "Contributors" of parts. Contributors may hold patents on the parts, but they promise not to assert any present or future proprietary rights against Users. Unlike copy-left

91 As previously mentioned, in this document, "commercially available" involves final products either available for sale or that are distributed through non-commercial and/or non-profit enterprises, and products approved for commercial release but which are not yet commercialized.

open-source software, Users have no obligation to openly share the devices or parts they make with the BioBricks. They can patent novel devices if they want to, meaning that they can build private, proprietary systems on the open platform (BioBricks Foundation, 2021; Calvert, 2012). As in open-source software, proponents consider this approach as more likely to lead to innovation as well as furthering transparency and openness (Calvert, 2012; van den Belt, 2013). Additionally, in 2018 the BioBricks Foundation launched the Open Material Transfer Agreement (OpenMTA) as a simple standardized legal tool intended to facilitate sharing of biomaterials on an open basis by researchers, institutions and broader communities, by relaxing restrictions on redistribution and commercial use (Kahl et al., 2018). "Open" alternatives to the patent system in a research and innovation context appear to be gaining greater traction internationally. For example, at the fortieth session of UNESCO's General Conference, in 2019, its 193 member States tasked the Organization with the development of an international standard-setting instrument on Open Science. Draft text of a UNESCO Recommendation on Open Science[92] will be put forward for adoption by UNESCO's General Conference during its next session, in November 2021. The Recommendation provides a framework to support scientific cooperation and to make science more transparent, accessible, equitable and inclusive. It includes definitions for a number of open elements including open science, open scientific knowledge and open research data, in which timely, free and open access to research data emerges as a foundational pillar of Open Science.

IP regimes for synthetic biology could have a variety of impacts on biodiversity and related considerations. In the USA, each patent application costs $10,000 (Henkel & Maurer, 2007). If patenting becomes established as the necessary method of claiming of IP rights on synthetic biology, the high cost could influence the kinds of applications of synthetic biology that are pursued (high-profit applications targeting wealthy populations), as well as the types of organizations (continuing the concentration of ownership and control in large transnational corporations) (ICSWGSB, 2011; ETC Group, 2007; Redford et al., 2013). If patent "thickets" form in certain areas of synthetic biology applications, this could also restrict its accessibility by less wealthy countries (Redford et al., 2013). One early example is LM "golden rice" — actually developed before the term synthetic biology was widely used — for which more than 70 patent rights needed to be cleared (Potrykus, 2001; Rutz, 2009). A concern of civil society groups is that strong IP regimes could also restrict access to information for carrying out independent, effective risk assessments (International Civil Society Working Group on Synthetic Biology (ICSWGSB), 2011). Finally, it is possible that an additional challenge for conservation biologists and synthetic biologists to work together could be that the types of biological knowledge used by synthetic biologists are "much more restricted" (Redford et al., 2013). As a counterbalance, industry perspectives must also be considered, particularly regarding the high costs and regulatory barriers associated with taking commercial applications to market, for which IP protection is argued to provide a necessary incentive to prevent free-riding and without which such investment would not occur (WIPO, 2004). The reader is directed to subsection 9.4.1 for further reading on other international instruments, in addition to the Convention on Biological Diversity, discussing intellectual property as they potentially relate to biodiversity.

8. Potential implications of the Convention and its Protocols for the governance of synthetic biology

8.1. Convention on Biological Diversity

8.1.1. Objectives of the Convention on Biological Diversity

The objectives of the Convention on Biological Diversity are the conservation of biological diversity, the sustainable use of its components, and the

92 Available at https://unesdoc.unesco.org/ark:/48223/pf0000378381.locale=en.

fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies (Article 1). The Convention text does not specifically refer to synthetic biology. However, synthetic biology falls within the scope of biotechnology, as defined by the Convention.[93] Depending on the scope of synthetic biology's definition (including the operational definition developed by the Ad Hoc Technical Expert Group on Synthetic Biology considered useful by the Conference of the Parties as a starting point for the purpose of facilitating scientific and technical deliberations under the Convention and its Protocols),[94] the provisions of the Convention most relevant to the governance of synthetic biology are outlined below in subsections 8.1.2 to 8.1.7.

As a general note, decisions of the Parties provide assistance in interpreting the provisions of the Convention. For example, the ecosystem approach – as embodied in the 12 complementary and interlinked principles whose application was recommended by the Conference of the Parties at its fifth meeting, in 2000, in decision V/6 – provides a strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way. Although not considered in detail herein, the ecosystem approach is noteworthy in relation to the objectives of the Convention as it constitutes the primary framework for action under the Convention whereby its application is designed to help to reach a balance of the three objectives of the Convention. The approach recognizes that humans, with their cultural diversity, are an integral component of ecosystems, and is based on the application of scientific reasoning, including traditional knowledge, to protect and manage the environment in order to resolve ecosystem issues.

8.1.2. Principle of the Convention (Article 3)

Article 3 of the Convention provides that "*States have, in accordance with the Charter of the United Nations and the principles of international law, the sovereign right to exploit their own resources pursuant to their own environmental policies, and the responsibility to ensure that activities within their jurisdiction or control do not cause damage to the environment of other States or of areas beyond the limits of national jurisdiction*". For a discussion of this principle in the context of synthetic biology techniques, see subsection 9.3.1(a) concerning the prevention of transboundary harm to the environment as an established principle under international customary law.

8.1.3. Impact assessment and minimizing adverse impacts (Article 14.1(a) and (b))

Article 14.1(a) of the Convention commits each Party to, as far as possible and as appropriate, "*introduce appropriate procedures requiring environmental impact assessment of its proposed projects that are likely to have significant adverse effects on biological diversity (…)*". Article 14.1(b) requires each Party, as far as possible and as appropriate, to "*introduce appropriate arrangements to ensure that the environmental consequences of its programmes and policies that are likely to have significant adverse impacts on biological diversity are duly taken into account*".

This provision requires Parties that do not have procedures for environmental impact assessments for their proposed projects, which are likely to cause significant adverse effects on biological diversity, to introduce such procedures (Glowka et al., 1994). Where synthetic biology projects are projects of a Party and are likely to have significant adverse effects on biological diversity, they should be covered by the environmental impact assessment procedures required by Article 14.1(a).

The Convention does not define further what is understood by "likely" and "significant". As elaborated in subsection 9.3.1(a), "significant" under international customary law could be understood to establish a *de minimis* threshold and to require a certain intensity of impact. Assessing the probability of

93 *"... any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use".*

94 As noted under "Scope and methods" (section A).

potential negative impacts of synthetic biology techniques may be challenging for many applications in light of doubts cast upon the adequacy of risk assessment methodologies for certain synthetic biology applications (section 6.1). In addition, interpretations of "likely" and "significant" may also have to take into account the case of low-probability, high-impact scenarios which some synthetic biology applications may pose (as noted further in subsection 9.3.1(a) in relation to the prevention of transboundary harm to the environment and the duty to undertake an environmental impact assessment).

8.1.4. Biosafety provisions associated with LMOs (Article 8(g) and 19(4))

The majority of the Convention's work on biosafety has focused on the negotiations that led to the Cartagena Protocol on Biosafety, in response to Article 19, paragraph 3, of the Convention and decision II/5 of the Conference of the Parties (SCBD, 2005). The Convention itself addresses biosafety through Article 8(g) and Article 19, paragraph 4.

Article 8(g) requires Parties, as far as possible and as appropriate, to "*establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health*". Article 19, paragraph 4 states that Parties shall provide any available information about their use and safety regulations in handling any LMO resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity, as well as any available information on the potential adverse impact of the specific organisms concerned to a Party into which those organisms are to be introduced.

"Biotechnology" is defined in Article 2 of the Convention as "*any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.*" According to the IUCN *Guide to the Convention on Biological Diversity* (Glowka et al., 1994), this definition was "*designed to include both present and future technologies and processes*". The Convention does not define "biological systems", "living organisms", or "derivatives thereof" (see Article 2).

Much of the synthetic biology research (see section 2) and most of its commercialized products (see section 3) involve the use of living organisms (or at least the use of biological systems or derivatives of biological systems or living organisms) and thus it would be classified as biotechnology as defined by the Convention.

The extent to which biosafety provisions of the Convention apply to synthetic biology depends on the interpretation of "living modified organisms resulting from biotechnology", "likely to have adverse environmental impacts" and "potential adverse impacts", and "use and release", which are discussed below.

(a) "Living modified organisms"

The text of the Convention does not define "living modified organisms". According to the IUCN guide to the Convention, negotiators of the Convention replaced the term "genetically modified organisms" with "living modified organisms" in order to broaden the scope of obligations under the relevant articles (Glowka et al., 1994). Unlike the Protocol's definition of LMOs, which applies to organisms obtained through the use of *modern* biotechnology (see subsection 8.2.1 below), the Convention's use of the term is meant to include organisms whose genetic material is modified through traditional techniques, such as selective breeding and artificial insemination, as well as "*organisms whose genetic material is more directly modified through, for example, recombinant DNA technology*" (Glowka et al., 1994).

The Convention does not define "living organisms" either; the Protocol defines "living organism" as "*any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids*" (Article 3(h) Cartagena Protocol on Biosafety). Whether an organism resulting from synthetic biology techniques would be considered

an LMO in the context of the Convention might depend on which products of synthetic biology are considered as "living". For example, virus-like macromolecular assemblies, protocells and naked DNA are unlikely to be considered as "living", as discussed further in subsection 8.2.1.

(b) "Are likely to have adverse environmental impacts"/ "potential adverse impacts"

Both Articles 8(g) and 19, paragraph 4, of the Convention use probability-based language – "*are likely to have adverse environmental impacts*" and "*potential adverse impacts*". An initial matter of interpretation is establishing the thresholds of probability for "likely" and "may". The IUCN guide to the Convention suggests that assessing the likelihood of risk could be guided by three primary criteria: (a) familiarity with the organism and its characteristics; (b) the organism's contemplated application; and (c) the environment into which the organism will or could be released (Glowka et al., 1994).

The Protocol may also be relevant in this regard. As considered further in subsection 8.2.2(a) below, according to its Article 15 and Annex III on risk assessment, the purpose of conducting a risk assessment under the Protocol is to identify and evaluate the "potential adverse effects" of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. As noted in subsection 9.3.1, there does not appear to be consensus among stakeholders, including scientists, academia, industry, civil society and IPLCs, on how well the potential adverse effects related to synthetic biology are known and can be assessed.

(c) Use and release of living modified organisms

Article 8(g) of the Convention addresses "risks associated with the use and release" of LMOs. One possible interpretation of this text is that *two* categories of risks are included – risks associated with the use of LMOs and risks associated with the release of LMOs. The text could also be interpreted to consider only those risks associated with both the use *and* release of LMOs.

Most synthetic biology products that are commercially available are only intended for use in contained, industrial or laboratory settings (see section 3.3), for example for biopharmaceuticals, carbon cycling, fabric, cosmetics/fragrances, and food and food ingredients resulting from synthetic metabolic engineering that perform specific industrial processes (such as enzymes to degrade biomass) or produce specific compounds (such as yeast producing artemisinic acid). More recently, products that are intended to be released in semi-managed, managed or urban settings have become commercially available, such as certain genome-edited soya beans, and nitrogen fertilizer based on engineered bacteria as per the examples noted in subsection 3.2.1. Products intended for release are anticipated to increase in the coming years, with field trials or near-market ready research spanning a broad range of applications, including certain self-limiting insects, engineered gene drives in mosquito for potential control of vector-borne diseases, among others, as further elaborated in subsection 3.2.2. Where such products are considered to be LMOs, risks associated with their use and release, as provided in Article 8(g), become relevant.

8.1.5. Access to genetic resources and benefit-sharing arising from their utilization (Article 15)

(a) Genetic resources for their use in synthetic biology[95]

While the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization details more precise obligations in relation to access and benefit-sharing for its Parties, Article 15 of the Convention continues to apply to all Parties to the Convention.

Article 15 recognizes the sovereign rights of States over their natural resources and provides that the authority to determine access to genetic resources rests with national governments and is subject to national legislation. It may be relevant to synthetic biology if it involves the access to genetic resources

95 It should be noted that this document is made available for the information of Parties to the Convention and is not intended to affect the rights and obligations of Parties to the Convention or its Protocols.

for use in synthetic biology processes and could give rise to an obligation to share benefits from the utilization of the genetic resources.

Furthermore, Article 15 includes the provisions that Parties shall endeavour to create conditions to facilitate access to genetic resources for environmentally sound uses by other Contracting Parties (paragraph 2); that granted access shall be on mutually agreed terms (paragraph 4) and subject to prior informed consent, unless otherwise determined by the Party providing the genetic resources (paragraph 5); and that "*Parties shall take legislative, administrative or policy measures … with the aim of sharing in a fair and equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Contracting Party providing such resources*" (paragraph 7).

In the cases where synthetic biology utilizes genetic resources and requires access to those resources, the access requirements of the Convention would, in general, apply and thus require prior informed consent (unless otherwise determined) and the negotiation of mutually agreed terms.

However, there are cases where it is not clear that the material accessed for its use in synthetic biology can be considered "genetic resources" or "genetic material" in accordance with the definitions contained in Article 2 of the Convention. The Convention defines "genetic resources" as genetic material of actual or potential value. Additionally, "genetic material" is defined as any material of plant, animal, microbial or other origin containing functional units of heredity.

Therefore, "genetic material" includes material from any origin so long as it contains "functional units of heredity". Functional units of heredity are not defined in the text of the Convention. Schei and Tvedt (2010) argue that because the definition refers to both actual and potential value, the word "functional" encompasses a dynamic element and the term "genetic material" can be interpreted in line with contemporary knowledge and technology. When the Convention was negotiated, the general understanding was that functional units of heredity distinguished genes from "junk" DNA. Today, however, scientific understandings of heredity have changed dramatically; junk DNA is no longer considered "junky", and functional units of heredity may need to be interpreted beyond the gene itself to include, for example, epigenetics, which involve functional, and sometimes inherited, changes in the regulation of gene activity and expression that are not dependent on gene sequence (Ganesan, 2018; Gemmell, 2021) and which are increasingly implicated in linking genetics to the environment and disease (Cavalli & Heard, 2019).

As said above, the Convention defines "genetic resources" as genetic material of actual or potential value. "Value" within the context of the Convention includes not just economic value, but also ecological, genetic, social, scientific, educational, cultural, recreational and aesthetic values (Preamble). Schei and Tvedt (2010) argue that because the definition refers to both types of value – actual and potential – it encompasses the state of art of technology as well as dynamic future realizations of value. Synthetic biology tools and techniques are aiding researchers in discovering new aspects of value in materials (Laird & Wynberg, 2012). Synthetic biology is opening up new ways to capture increased value from genetic materials, and thus may affect Parties' interpretations of the definitions of "genetic resources" and "genetic material" as contained in the Convention and, by reference, the Nagoya Protocol.

For example, synthetic biology relies heavily on digital information on functional units of heredity, such as specific DNA sequences, and reflects a growing trend in research away from physical transfers of biological material and towards electronic transfers and use of digital information, a trend that has accompanied the rise of biotechnology more broadly and has accelerated further with modern synthetic biology tools and techniques (Houssen et al., 2020; Oldham, 2004). In an increasing array of contexts, researchers utilize information about the genetic composition – from DNA and RNA sequences to amino acid and protein sequences through to biochemical information – instead of the physical genetic resource. In

practice, however, the use of such information typically complements rather than supplants the use of a physical genetic resource. For example, although the costs and technical difficulty of DNA synthesis are rapidly decreasing, technology has yet to advance to enable the ready synthesis of entire organisms other than certain viruses, in which case, access to physical genetic resources will still be required, such as for the testing of the efficacy of medical countermeasures, including diagnostics, antivirals and vaccines, where synthesis costs are presently prohibitive or where certain synthesis methods are protected by intellectual property (Rourke et al., 2020). As a result of these developments, the issue of "digital sequence information on genetic resources"[96] was raised in 2016 during the thirteenth meeting of the Conference of the Parties, and in decision XIII/16, the Conference of the Parties decided to consider, at its fourteenth meeting, any potential implications of the use of digital sequence information on genetic resources for the three objectives of the Convention. A complementary decision was adopted at the second meeting of the Parties to the Nagoya Protocol (decision NP-2/14).

At its fourteenth meeting, in 2018, the Conference of the Parties adopted decision 14/20, which noted that "*as there is a divergence of views among Parties regarding benefit-sharing from the use of digital sequence information on genetic resources, Parties commit to working towards resolving this divergence through the process established in the present decision, with the aim of strengthening the fulfilment of the third objective of the Convention and Article 15, paragraph 7, without prejudice to the circumstances in which this article applies*" (paragraph 6). The process established in the decision included the submission of views, the commissioning of studies and work by an ad hoc technical expert group (AHTEG). The outcomes of the AHTEG are to be considered by the Open-ended Working Group on the Post-2020 Global Biodiversity Framework, which is to make recommendations to the Conference of the Parties at its fifteenth meeting on how to address digital sequence information on genetic resources in the context of the post-2020 global biodiversity framework.[97]

The AHTEG met in March 2020 and, *inter alia*, developed options for operational terms and their implications to provide conceptual clarity on digital sequence information on genetic resources, and also identified key areas for capacity-building.[98] At the time of writing, the meeting of the Open-ended Working Group on the Post-2020 Global Biodiversity Framework at which the outcomes of the AHTEG were to be considered was still to be held.

The issue of digital sequence information is also being discussed in other international fora related to genetic resources, including the International Treaty on Plant Genetic Resources for Food and Agriculture, the Pandemic Influenza Preparedness (PIP) Framework of WHO, and negotiations towards an international agreement on the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction under UNCLOS, each of which are likely to consider the outcomes of the process under the Convention and its Nagoya Protocol to inform their own deliberations.

(b) Genetic resources originating from synthetic biology

Another open question is whether the components, organisms and products resulting from synthetic biology can be considered "genetic resources" under the Convention. Different areas of synthetic biology research may raise different considerations regarding whether they constitute genetic resources within the definition of the Convention. For example, taking into consideration some of the areas of research

96 The Conference of the Parties has also noted that the term "digital sequence information" may not be the most appropriate term and it is used as a placeholder until an alternative term is agreed.

97 The Parties to the Nagoya Protocol also adopted, at their third meeting, a decision on digital sequence information on genetic resources in which they requested the Open-ended Working Group on the Post-2020 Global Biodiversity Framework to submit the outcome of its deliberations for consideration by the Parties to the Protocol at their fourth meeting; see decision NP-3/12.

98 In evaluating the scope of digital sequence information on genetic resources and terminology, the AHTEG on DSI noted that clearly defined groups would assist negotiators in the Convention process and other forums when discussing topics related to digital sequence information, and proposed a conceptual approach for defining such groups; see the report of the AHTEG, CBD/DSI/AHTEG/2020/1/7.

that are considered synthetic biology as identified in section 2 above:

- *DNA-based parts and devices, synthetic metabolic pathway engineering, and genome-level engineering* – These areas of research involve designing and synthesizing stretches of DNA, RNA, and whole genomes. The organisms resulting from these synthetic biology techniques contain DNA. However, the products these organisms are sometimes designed to create, such as pharmaceutical molecules and fuel, generally do not contain DNA.

- *Protocell construction* – Protocell research aims to create the simplest possible components to sustain reproduction, self-maintenance and evolution (Lam et al., 2009; Solé et al., 2007). Protocell designs usually contain some kind of information-carrying molecule; these could possibly be understood to functionally operate as "units of heredity". However, some protocell research is attempting to develop cells without the ability to evolve or replicate (Ma & Feng, 2015; Presidential Commission for the Study of Bioethical Issues, 2010). Depending on the meaning of functional units of heredity, such cells may not fall within the definition of "genetic material".

- *Xenobiology* – This research focuses on altering the basic form of nucleic and amino acids, for example by creating nucleic acids with novel bases or novel backbones which are not found in nature. Whether this would be considered "genetic material" depends on whether XNA and other modified forms of information-carrying molecules would be considered to operate as functional units of heredity. These organisms may still be able to reproduce themselves, however, so they may be understood to contain functional units of heredity.

The consideration of the components, organisms and products resulting from synthetic biology as genetic resources within the context of the Convention could lead to some questions regarding the application of the principle of state sovereignty over genetic resources and access and benefit-sharing obligations as well as the application of the Convention's provisions regarding the conservation and sustainable use of biodiversity.

8.1.6. Technology transfer and cooperation (Articles 16 to 19)

A number of decisions of the Conference of the Parties (e.g. decisions XI/29, XII/2 B, XIII/23 B and 14/24) have sought to elaborate on technical and scientific cooperation and technology transfer based on Articles 16 to 19 of the Convention. These have been implemented through various partnerships, programmes and initiatives, including the Global Taxonomy Initiative, the Bio-Bridge Initiative, the Forest Ecosystem Restoration Initiative, the Sustainable Ocean Initiative, the Global Partnership for Plant Conservation, the Collaborative Partnership on Sustainable Wildlife Management, and the Inter-agency Liaison Group on Invasive Alien Species (SCBD, 2020a, 2020c). Further, pursuant to decisions XIII/23 and 14/24 relating to capacity-building, technical and scientific cooperation and technology transfer, the Executive Secretary has initiated a process for renewing and reviewing technical and scientific cooperation and for preparing a draft long-term strategic framework for capacity-building beyond 2020 aligned with the draft post-2020 global biodiversity framework and the 2030 Agenda for Sustainable Development.

Article 16, paragraph 1 provides that each Party will undertake "*to provide and/or facilitate access for and transfer to other Contracting Parties of technologies that are relevant to the conservation and sustainable use of biological diversity or make use of genetic resources and do not cause significant damage to the environment*". Article 16 explicitly includes "biotechnology" in the provisions on access to and transfer of technology (Article 16, paragraph 1). As discussed in subsections 8.1.4 and 8.2.1, technologies associated with synthetic biology may fall under the definition of biotechnology.

Technologies associated with synthetic biology may fulfil both criteria set out in Article 16, paragraph 1: (a) be of relevance to conservation and sustainable use of biodiversity; and (b) use genetic resources

and not cause significant damage to the environment. Case-by-case assessments would be needed to determine how these criteria apply to specific technologies. Considering the first criterion, some areas of synthetic biology research do aim to produce applications relevant to conservation and sustainable use, for instance, as per the research examples in subsection 3.1.3 concerning research applications of synthetic biology in bioremediation, control of vector borne diseases in biodiversity conservation efforts and improving resilience in wild animal and plant populations. Considering the second criterion, much of synthetic biology research could be considered to "make use of genetic resources"; however, whether or not specific synthetic biology technologies cause significant damage to the environment would require an impact assessment.

Developing countries are to be provided "fair and most favourable terms" to access to and transfer of technologies (Article 16, paragraph 2) that "*are relevant to the conservation and sustainable use of biological diversity or make use of genetic resources and do not cause significant damage to the environment*" (Article 16, paragraph 1). Article 19 also specifically addresses developing countries, obliging Parties to "*take all practicable measures to promote and advance priority access on a fair and equitable basis by Contracting Parties, especially developing countries, to the results and benefits arising from biotechnologies based upon genetic resources provided by those Contracting Parties*" (Article 19, paragraph 2), and that Parties shall "*provide for the effective participation in biotechnological research activities by those Contracting Parties, especially developing countries, which provide the genetic resources for such research, and where feasible in such Contracting Parties*" (Article 19, paragraph 1; see also Article 15, paragraph 6). Scientific publications provide a useful proxy indicator for R&D activities around the world. Information presented earlier (section 1) on the origin of synthetic biology publications and research and development showed that there is a concentration of R&D activities in developed countries. This suggests an opportunity to promote technology transfer and technical and scientific cooperation with developing countries in order to address the technology gap in relation to synthetic biology, including through the long-term strategic framework for capacity-building beyond 2020 mentioned above.

Some organizations are undertaking capacity-building initiatives with a specific focus on synthetic biology. These include the Building International Capacity in Synthetic Biology Assessment and Governance (BICSBAG) Project[99] and CABANA.[100] The iGEM Competition (see section 1.10 and subsection 7.3.4) can also be seen as capacity-building and technology transfer in the field of synthetic biology.

8.1.7. Provisions related to indigenous peoples and local communities (Articles 8(j) and 10(c))

The preamble to the Convention recognizes "*the close and traditional dependence of many indigenous and local communities embodying traditional lifestyles on biological resources, and the desirability of sharing equitably benefits arising from the use of traditional knowledge, innovations and practices relevant to the conservation of biological diversity and the sustainable use of its components*".

Substantive provisions of the Convention that are most relevant to IPLCs include Article 8, which addresses *in situ* conservation, and Article 10, which addresses sustainable use of components of biological diversity. Specifically, Article 8(j) provides that each Party shall, as far as possible and as appropriate, "*Subject to its national legislation, respect, preserve and maintain knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity and promote their wider application with the approval and involvement of the holders of such*

99 The BICSBAG Project is a capacity-building initiative coordinated by the African Centre for Biodiversity, the ETC Group, and the Third World Network. It is accessible at https://www.synbiogovernance.org/about/.

100 A capacity strengthening project focused on bioinformatics in Latin America, funded by the UK Government; see https://www.cabana.online/.

knowledge, innovations and practices and encourage the equitable sharing of the benefits arising from the utilization of such knowledge, innovations and practices". Additionally, under Article 10(c), Contracting Parties are required, as far as possible and as appropriate, to "*protect and encourage customary use of biological resources in accordance with traditional cultural practices that are compatible with conservation or sustainable use requirements*".

The Parties have approved guidelines and other tools to facilitate the implementation of Article 8(j) and related provisions. A number of these address the prior informed consent of IPLCs and so are particularly relevant to societal concerns arising from the application of synthetic biology research, as considered in subsection 5.1.2 concerning IPLCs specifically, and also section 5.3 concerning ethical concerns more generally. Notably, these guidelines and other tools include:

- The Akwé: Kon Voluntary Guidelines for the Conduct of Cultural, Environmental and Social Impact Assessments Regarding Developments Proposed to Take Place on, or which are Likely to Impact on, Sacred Sites and on Lands and Waters Traditionally Occupied or Used by Indigenous and Local Communities, adopted by the Conference of the Parties at its seventh meeting, in 2004 (SCBD, 2004);

- The Tkarihwaié:ri Code of Ethical Conduct to Ensure Respect for the Cultural and Intellectual Heritage of Indigenous and Local Communities Relevant for the Conservation and Sustainable Use of Biological Diversity, adopted by the Conference of the Parties at its tenth meeting, in 2010 (SCBD, 2011);

- The Mo'otz Kuxtal Voluntary Guidelines for the development of mechanisms, legislation or other appropriate initiatives to ensure the "prior and informed consent", "free, prior and informed consent" or "approval and involvement", depending on national circumstances, of indigenous peoples and local communities for accessing their knowledge, innovations and practices, for fair and equitable sharing of benefits arising from the use of their knowledge, innovations and practices relevant for the conservation and sustainable use of biological diversity, and for reporting and preventing unlawful appropriation of traditional knowledge, adopted by the Conference of the Parties at its thirteenth meeting, in 2016 (SCBD, 2019).

More recently, at its fourteenth meeting, in 2018, the Conference of the Parties explicitly called for free, prior and informed consent, or approval and involvement of potentially effected IPLCs to be sought or obtained in relation to the introduction of LMOs containing engineered gene drives into the environment, including for experimental releases and research and development purposes – where appropriate and applicable in accordance with national circumstances and legislation (decision 14/19).

Issues pertaining to IPLCs have also featured prominently in the expert groups established by the Parties to evaluate issues related to synthetic biology under the Convention, as described further in subsection 8.1.8 below. For example, the experts appointed to the AHTEG on Synthetic Biology convened in 2019 included two experts nominated by IPLC organizations. The participation of IPLCs in activities related to synthetic biology carried out under the Convention was acknowledged by the 2019 AHTEG as important for building the necessary understanding for informed consideration. Together with appropriate communication and engagement with communities, it enables them to engage in the assessment of actual and potential impacts of synthetic biology. This built on an acknowledgement by the 2017 AHTEG that IPLCs regarded all components of Mother Nature as living entities and the potential for synthetic biology to impact cultural values and principles, including the relationship of IPLCs with Mother Nature, as well as noting that the development of synthetic biology technologies "*should be accompanied by the full and effective participation of indigenous peoples and local communities*".

8.1.8. *Decisions of the Conference of the Parties referring to synthetic biology*

The evolving nature of international deliberations concerning synthetic biology applications, including growing awareness and interest in the actual and potential implications of such applications to biodiversity and the objectives of the Convention, are evident in the decisions of the Conference of the Parties.

The first decisions by the Conference of the Parties referring directly to synthetic biology were adopted by it in 2010. Since then, it has adopted a number of decisions which raise substantive issues related to synthetic biology as summarized in table 2 below.

These incremental decisions have driven a steady build-up in intersessional activities focused on synthetic biology. At its tenth meeting, the Conference of the Parties commenced a process of information gathering through invitations for the submission of information on synthetic biology, which has been repeated for subsequent meetings of the Conference of the Parties, initially for consideration by SBSTTA and subsequently to inform the deliberations of the AHTEG on Synthetic Biology, which was first established by the Conference of the Parties in decision XII/24 and whose mandate has been extended at two subsequent meetings to date. An open-ended online forum to support the work of the AHTEG has also been extended biennially. Documents related to synthetic biology, such as submissions of information, AHTEG and online forum reports, are available through the online portal on synthetic biology.[101] The outcomes of the AHTEG on Synthetic Biology are considered at meetings of SBSTTA, whose recommendations form the basis of draft decisions that are negotiated at subsequent meetings of the Conference of the Parties. Additionally, acknowledging that the provisions of the Cartagena Protocol on Biosafety may also apply to living organisms resulting from synthetic biology, the Parties to the Convention and the Protocol have implemented a coordinated approach on the issue of synthetic biology.[102]

8.2. *Cartagena Protocol on Biosafety*

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity was negotiated further to Article 19(3) of the Convention. The Protocol applies to the transboundary movement, transit, handling and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health (Article 4; Cartagena Protocol on Biosafety). Article 1 of the Protocol explicitly refers to the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development. The Protocol entered into force in 2003 and had 173 Parties as of March 2021.

This section examines various elements that could play a role in determining which organisms or products developed using synthetic biology might be considered as LMOs in the context of the Protocol. Risk assessments undertaken pursuant to the Protocol must be carried out in accordance with Annex III as specified in Article 15; the general principles, methodology, and points to consider of Annex III are examined for application to synthetic biology.

8.2.1. *LMOs and components, organisms and products of synthetic biology*

The Protocol defines LMOs as "*any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology*" (Article 3(g); Cartagena Protocol on Biosafety). To be considered LMOs, the applications of synthetic biology would thus have to: (a) be a living organism, (b) possess a novel combination of genetic material; and (c) result from the use of modern biotechnology. It should be stressed that these terms are intrinsically interlinked. For example, a novel combination of genetic material that did not result from the use of modern biotechnology would not be considered an LMO in the context of the Protocol.

The AHTEG on Synthetic Biology has considered the question of synthetic biology organisms that may fall outside the definition of "living modified organism" in the Cartagena Protocol on Biosafety.

101 https://bch.cbd.int/synbio/.
102 Decisions XII/24, 14/19, BS-VII/12 and CP-9/13.

Table 2. Summary of substantive issues related to synthetic biology arising from decisions of the Conference of the Parties to the Convention on Biological Diversity at its tenth to fourteenth meetings

Decision	Title	Substantive issues
X/13, para. 4 (2010)	New and emerging issues	• Invited Parties, other Governments and relevant organizations to apply the precautionary approach to the field release of synthetic life, cell or genome into the environment.
X/37, para. 16 (2010)	Biofuels and biodiversity	• Urged the application of the precautionary approach to the introduction and use of LMOs in biofuel production as well as to the field release of synthetic life, cell, or genome into the environment, acknowledging the entitlement of Parties to suspend the release of synthetic life, cell, or genome into the environment.
XI/11 paras. 3 and 4 (2012)	New and emerging issues	• Noted the need to consider the potential impacts of components, organisms and products resulting from synthetic biology techniques on the conservation and sustainable use of biological diversity and associated social, economic and cultural considerations; • Recognizing the development of technologies associated with synthetic life, cells or genomes, and the scientific uncertainties of their potential impact on the conservation and sustainable use of biological diversity, urged Parties and invited other Governments to take a precautionary approach, in accordance with the preamble of the Convention and with Article 14, when addressing threats of significant reduction or loss of biological diversity posed by organisms, components and products resulting from synthetic biology.
XI/27, para. 6 (2012)	Biofuels and biodiversity	• Urged Parties and other Governments to monitor the rapidly developing technology associated with biofuels and to apply the precautionary approach.
XII/24 (2014)	New and emerging issues: synthetic biology	• Urged Parties and invited other Governments to take a precautionary approach, in accordance with paragraph 4 of decision XI/11, in relation to technologies associated with synthetic life, cells or genomes; • Urged Parties and invited other Governments to approve organisms resulting from synthetic biology techniques for field trials only after appropriate risk assessments have been carried out.
XIII/17 (2016)	Synthetic biology	• Reaffirmed decision XII/24, in which it urged Parties and invited other Governments to take a precautionary approach, in accordance with decision XI/11, paragraph 4; • Reiterated paragraph 3 of decision XII/24 and noted that it can also apply to some LMOs containing engineered gene drives; • Acknowledged the operational definition of synthetic biology as an outcome of the AHTEG on Synthetic Biology and considered it useful as a starting point for the purpose of facilitating scientific and technical deliberations under the Convention and its Protocols; • Noted that the general principles and methodologies for risk assessment under the Cartagena Protocol on Biosafety and existing biosafety frameworks provide a good basis for risk assessment regarding living organisms developed through current applications of synthetic biology, or that are currently in the early stages of research and development, but such methodologies may need to be updated and adapted for current and future developments and applications of synthetic biology; • Welcomed the recommendation of the Conference of the Parties serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety, in its decision BS-VII/12, on a coordinated approach on the issue of synthetic biology, taking into account that the provisions of the Protocol may also apply to living organisms resulting from synthetic biology, and invited it to take into account in its future deliberations relevant information resulting from processes under the Convention.
14/19 (2018)	Synthetic biology	• Agreed that horizon scanning, monitoring and assessing of technological developments is needed for reviewing potential impacts of synthetic biology; • Called upon Parties and other Governments, taking into account the current uncertainties regarding engineered gene drives, to apply a precautionary approach. • Called upon Parties and other Governments to only consider introducing LMOs containing an engineered gene drive into the environment when: » Scientifically sound case-by-case risk assessments have been carried out; » Risk management measures are in place to avoid or minimize potential adverse effects, as appropriate; » Where appropriate, the free, prior and informed consent, or approval and involvement, of potentially affected IPLCs is sought or obtained.

In the report of its 2019 meeting, the AHTEG noted that both legal and technical considerations inform the question of whether a synthetic biology organism falls within or outside the Protocol's definition of "living modified organism".

It discussed a number of examples of synthetic biology organisms that may fall outside the definition of "living modified organism" and acknowledged that virus-like macromolecular assemblies and protocells were not LMOs as they do not constitute living organisms (see subsection 8.2.1(a) below). There were different views on whether organisms whose genomes had been edited without the use of nucleic acids using only protein reagents introduced into the cell, for example by ZFN/TALEN/MN applications, would fall under the definition of "living modified organism". The AHTEG considered that it was unclear whether some transiently modified organisms would constitute LMOs as defined in the Protocol.

In 2019, the AHTEG on Synthetic Biology recalled the conclusion in its 2017 report that most living organisms already developed or currently under research and development through techniques of synthetic biology fell under the definition of LMOs as per the Protocol. It agreed that this conclusion was still valid (SCBD, 2019). It further noted, however, that given the rapid developments in the field, it may be possible that synthetic biology organisms developed in the future could fall outside the definition of "living modified organism" in the Protocol. Were such a situation to arise, it was recognized that the relevant obligations in the Convention would continue to apply (see also subsection 8.1.4 above).

Examining the three elements of the definition of LMO in the Cartagena Protocol on Biosafety as outlined above can help to see how it relates to applications of synthetic biology.

(a) Living organisms

The Protocol defines a "living organism" as "*any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids*" (Article 3(h); Cartagena Protocol on Biosafety). "Genetic material" is not defined in the Protocol; in the Convention it is defined as any material "containing functional units of heredity" (Article 2). Given this definition, many areas of research in synthetic biology would be considered as producing living organisms, including microbes produced by genome-level engineering and cells altered by synthetic metabolic engineering (see sections 2.3 and 2.4 above).

Two outstanding questions regarding the scope of living organisms in relation to current uses of synthetic biology are (a) products of organisms resulting from synthetic biology techniques; and (b) naked DNA and constituent parts.

Products of organisms resulting from synthetic biology techniques

According to the IUCN *Explanatory Guide to the Cartagena Protocol on Biosafety*, the products of LMOs (referred to as "products thereof") were extensively discussed during the negotiations of the Protocol (Mackenzie et al., 2003). "Products thereof" in the context of the Protocol seem to primarily refer to LMOs that have been processed. They are included in notifications under Annex I and risk assessments under Annex III if they contain "detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology" (Article 20, paragraph 3(c); Annex I, paragraph (i); and Annex III, paragraph 5; Cartagena Protocol on Biosafety).

Organisms resulting from synthetic biology techniques that are currently used for commercial purposes are largely microorganisms that have been altered to produce specific compounds, such as specialized chemicals, fuels, flavours, and pharmaceuticals (Wellhausen & Mukunda, 2009). The compounds are not simply processed LMOs; they are the by-products of microbes or microbial fermentation of biomass. They may fall within the Protocol's concept of "products thereof" if they contain nucleic acids containing a novel combination of genetic material. However, products that are in commercial use, such as vanillin and artemisinic acid, are

generally highly refined and would not be expected to contain nucleic acids.

DNA and constituent parts

The situation is less clear with regard to DNA and constituent parts. The Protocol's provisions on risk assessment and the minimum required information to be included in notifications under some of the Protocol's procedures may apply to naked DNA and its constituent parts resulting from synthetic biology techniques if they contain "*detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology*" (Annex I(i); and Annex III, paragraph 5; Cartagena Protocol on Biosafety).

In practice, many countries do not apply the Protocol's provisions on risk assessment and the minimum required information to naked DNA and its constituent parts because they are considered to be components rather than products of LMOs.

Furthermore, according to the IUCN *Explanatory Guide to the Cartagena Protocol on Biosafety,* the consensus decision was to not directly include plasmids or DNA in the Article 3(h) definition of living organisms (Mackenzie et al., 2003). DNA and parts produced for synthetic biology have been transported through postal mail for decades. For example, New England BioLabs Inc. offers the DNA Assembly Kit for sale over the internet. Components of the kit include destination plasmids and the upstream and downstream parts as purified DNA.[103] Purified, synthetic DNA from commercial DNA synthesis firms is available in a lyophilized (freeze-dried) form, typically as linear fragments (< 2 kilobases) or cloned into plasmids for larger fragments (Hughes & Ellington, 2017). Since the DNA is not inserted into living cells for shipment, the "naked" DNA and parts would likely not qualify as "living organisms" under the Protocol.

(b) Novel combination

A "novel combination of genetic material" is not a defined term. One interpretation is that it can result from a novel form or a novel arrangement of the functional units of heredity, regardless of whether or not this leads to a phenotypic change (Mackenzie et al., 2003). Supporting technologies of synthetic biology (see section 1) can be applied to produce novel genetic materials (Ren et al., 2020; Simon et al., 2019). For example, organisms resulting from synthetic biology techniques modelled after natural organisms such as the reconstructed HPXV (Noyce et al., 2018), karyotype engineered yeast (J. Luo et al., 2018; Shao et al., 2018) and the JCVI-syn3.0 strain (Hutchison et al., 2016) (see section 2.4 and subsection 3.3.3) are not exact copies of the originals, and thus may qualify as novel. The use of directed evolution, multiplex automated genome engineering, and genome editing techniques that do not incorporate new genetic material, may still be considered to result in "novel combinations" where they rearrange or extensively change existing genetic material, as in the case of gene shuffling (Magocha et al., 2018; Simon et al., 2019; X. Zhang et al., 2020).

(c) Modern biotechnology

"Modern biotechnology" is defined in the Protocol as:

> "*the application of:*
>
> *a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or*
>
> *b. Fusion of cells beyond the taxonomic family,*
>
> *that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection*" (Article 3(i); Cartagena Protocol on Biosafety).

The negotiators of the Protocol recognized that new techniques for modifying genetic information would continue to be developed (Mackenzie et al., 2003). According to the IUCN explanatory guide, although the definition gives two specific examples of *in vitro* nucleic acid techniques, other techniques

103 https://international.neb.com/products/dna-assembly-cloning-and-mutagenesis-kits/dna-assembly-cloning-and-mutagenesis-kits.

cannot be excluded from the definition so long as they overcome natural physiological reproductive or recombination barriers and are not techniques used in traditional breeding and selection. In a recent publication (Keiper & Atanassova, 2020), it was indicated that if the Protocol's definition of "modern biotechnology" was strictly applied to take into account the need for overcoming "*natural physiological or reproductive or recombination barriers and that are not techniques used in traditional breeding and selection*", some recombinant DNA and "new" technologies (e.g. genome editing) may be excluded from its scope. However, others are of the impression that genome editing techniques are not techniques used in traditional breeding and selection, and that genome-edited organisms are generated through the use of modern biotechnology techniques that bypass natural reproductive or recombination barriers, with genome editing allowing for modifications that would not otherwise naturally arise (Kawall, 2019; Sirinathsinghji, 2020; Heinemann et al., 2021).

8.2.2. *Key provisions of the Cartagena Protocol on Biosafety governing LMOs and related exemptions and exclusions*

The Protocol applies to the transboundary movement, transit, handling and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health (Article 4 Cartagena Protocol on Biosafety). This subsection provides an overview of the Protocol's provisions regarding risk assessment, the advance informed agreement (AIA) procedure including limited exemptions of some LMOs to some of the AIA provisions, and the exclusion of certain pharmaceuticals which are explicitly excluded from the scope of the Protocol. Only aspects relevant within the context of the applications of synthetic biology are indicated. For a detailed description of the provisions, reference is made to the Protocol.

(a) Risk assessment (Article 15 and Annex III)

Under Article 15, paragraph 2, of the Protocol, a risk assessment must be carried out for a Party of import to make a decision as per Article 10 for an intentional transboundary movement of an LMO to proceed (Article 10 and Article 15, paragraph 2, Cartagena Protocol on Biosafety). Risk assessments must be "*carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognized risk assessment techniques*" (Article 15, paragraph 1 Cartagena Protocol on Biosafety). According to Article 15 and Annex III, the purpose of conducting a risk assessment under the Protocol is to identify and evaluate the "potential adverse effects" of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health.

Although LMOs produced through synthetic biology may present characteristics that are not common to all LMOs, Annex III to the Protocol, including its general principles, points to consider and methodology, is still fully applicable to LMOs produced through synthetic biology and may also apply to "products thereof" that contain "*detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology*" (Article 20, paragraph 3(c); Annex I(i); and Annex III, paragraph 5; Cartagena Protocol on Biosafety). In addition, it could be discussed whether the risk assessment process of Annex III, which is based on the characteristics of the recipient and donor organisms and the added traits, might be adequate for synthetic biology organisms that have been developed to include genetic material from several donor organisms that may have also been optimized. In these cases, there might not be an appropriate comparator.

For certain categories of LMOs developed through synthetic biology, questions have been raised concerning how to apply the risk assessment procedures of the Protocol. These questions have focused on challenges with the long-established comparator approach, and knowledge gaps regarding assessment of ecological impacts where the application is unprecedented.

In decision CP-9/13 in 2018, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety recognized the

divergence in views among Parties on whether or not additional guidance on specific topics of risk assessment is needed. It decided to establish a process for the identification and prioritization of specific issues regarding risk assessment of LMOs, with a view to developing further guidance on risk assessment on the specific issues identified, and to consider, at its tenth meeting, whether additional guidance materials on risk assessment are needed for LMOs containing engineered gene drives, among other topics. To assist this process, decision CP-9/13 established an Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and extended the online forum on risk assessment and risk management in order to assist the AHTEG. The outcomes of the AHTEG concerning the need for guidance to be developed on risk assessment related to these LMOs are to be considered by the Subsidiary Body on Scientific, Technical and Technological Advice, which is to make a recommendation for consideration by the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety at its tenth meeting. At the time of writing, the meetings of the Subsidiary Body on Scientific, Technical and Technological Advice and of the Parties to the Protocol had not yet been held.

(b) Advanced informed agreement (AIA) provisions and related exemptions

The AIA is the central procedural mechanism set out in the Protocol to regulate transboundary movement of LMOs. The AIA procedure essentially requires that before the first transboundary movement of an LMO that is subject to the AIA procedure, the Party of import is notified of the proposed transboundary movement and is given an opportunity to decide whether or not the import shall be allowed and upon what conditions. This decision must be based upon a risk assessment as described in the section above.

Article 7 establishes the scope of the application of the AIA procedure – i.e. to which transboundary movements the procedure applies – and the AIA procedure itself is then set out in Article 8, 9, 10 and 12. The focus of the AIA procedure is on the first intentional transboundary movements of LMOs for intentional introduction into the Party of import. There are limited exemptions to the requirements of the AIA procedure.

"Contained use" (Article 6)

Under the Protocol, provisions for advanced informed agreement (AIA) do not apply to the transboundary movement of LMOs "*destined for contained use undertaken in accordance with the standards of the Party of import*" (Article 6, paragraph 2; Cartagena Protocol on Biosafety).[104] Contained use is defined as an operation "*undertaken within a facility, installation or other physical structure*" in which the contact of LMOs with, and impact on, the external environment is "effectively limit(ed)" by "specific measures" (Article 3(b); Cartagena Protocol on Biosafety). Negotiations on this topic concentrated on whether chemical or biological barriers could be considered as sufficient containment, or whether physical containment was necessary (Mackenzie et al., 2003; van der Meer, 2002). Ultimately, the text focuses on the *effectiveness* of containment measures, rather than the type of measure. The question of degree and quality of effectiveness is also left up to the Party to determine (Mackenzie et al., 2003). In decision CP-9/12, the Parties to the Protocol were reminded that intentional introduction into the environment can include introduction both for experimental or for commercial purposes, and that a field trial, confined field trial or experimental introduction is to be regarded as intentional introduction into the environment when the conditions specified in Article 3, paragraph b, of the Protocol are not met.

Some civil society groups consider there is a general lack of international regulations or standards concerning contained use and that this constitutes a major gap, including in relation to oversight of laboratory research (Lim & Lim, 2019) (note that containment in a health context by WHO is considered further in subsection 9.2.1). This concern arises especially because of the potential for unintentional releases of synthetic biology organisms, in particular LMOs containing engineered gene drives,

104 The Cartagena Protocol on Biosafety does not require that Parties regulate such LMOs according to the AIA provisions, but Parties are still free to use national legislation to require AIA and risk assessment (Mackenzie et al., 2003).

that might result in transboundary movement or the crossing of national borders, requiring an international response. Some issues have also been raised by some civil society groups specifically in relation to synthetic biology and the "contained use" AIA exemption. The International Civil Society Working Group on Synthetic Biology (ICSWGSB; 2011) argues that containment facilities that Parties consider to effectively contain LMOs may be unsuitable to contain organisms resulting from synthetic biology techniques.[105] They note a general assumption that physical containment of synthetic organisms is not practical, especially within large-scale production systems, and suggest that importing countries may need advance information in order to "*judge the effectiveness of available containment*" (*ibid*). This assumption is not universally shared; however, it does echo general biosafety concerns associated with synthetic biology applications considered in section 6. See also subsection 8.2.3(e) concerning handling transport, packaging and identification requirements applicable to contained use.

A second issue is whether specific members of the synthetic biology community should be considered able to provide for "contained use". EcoNexus, a European civil society group, does not consider DIY Bio (do-it-yourself biology)/citizen science individuals and collectives as being able to provide for "contained use" and is concerned that AIA "might become close to impossible" in such instances (EcoNexus, 2011). Conversely, different reports on DIY Bio found that few DIYers are using "sophisticated" synthetic biology, and most work in labs that are rated as Biological Safety Level 1, in a transparent and responsible manner (Grushkin et al., 2013; Kuiken, 2016; Landrain et al., 2013; Seyfried et al., 2014). Several developments involving self-regulation by the scientific community which are relevant to the DIY Bio discussion are considered in section 7.3.

A third and more general issue, which is not limited to LMOs produced by synthetic biology, is the potential to use the Protocol's provisions on contained use to circumvent the advance informed agreement procedure, for example if a laboratory imports a synthetic biology LMO for contained use and then makes a domestic application to release the LMO from containment (ICSWGSB, 2011). In such a situation, the process and standards for making a decision on releasing an LMO from containment would be based on a country's own rules and procedures as the Protocol's advance informed agreement procedure for decision-making based on a risk assessment in accordance with Annex III to the Protocol does not apply. This could have adverse implications for biosafety, for example, if domestic standards for risk assessment may be lower than the minimum provided in the Protocol's Annex III (ICSWGSB, 2011).

LMOs "intended for direct use as food or feed, or for processing" (Article 11)

The AIA procedure does not apply to the transboundary movement of LMOs intended for direct use as food or feed, or for processing (LMO-FFPs), although developing country Parties or Parties with an economy in transition may, in the absence of a domestic regulatory framework, declare through the Biosafety Clearing-House (BCH) that their decision prior to the first import of an LMO-FFP will be taken according to a risk assessment and a decision made within a predictable timeframe (Article 7, paragraph 2, and Article 11, paragraph 6; Cartagena Protocol on Biosafety). Furthermore, a Party that makes a final decision regarding domestic use of an LMO that may be subject to transboundary movement for direct use as food or feed, or for processing is to inform Parties through the Biosafety Clearing-House and this information is to include a risk assessment report consistent with Annex III to the Protocol (Article 11, paragraph 1 and Annex II (j); Cartagena Protocol on Biosafety). LMO-FFPs must be accompanied by documentation that "*clearly identifies that they 'may contain' living modified organisms and are not intended for intentional introduction into the environment*" (Article 18, paragraph 2(a); Cartagena Protocol on Biosafety).

105 This concern is premised on the ICSWGSB's view that organisms resulting from synthetic biology techniques, such as *de novo* organisms designed and constructed in the lab, may be significantly different from other organisms, including conventionally genetically-modified organisms, in that they lack analogues in the natural world (ICSWGSB, 2011).

(c) Exclusion from provisions of the Cartagena Protocol on Biosafety: pharmaceuticals for humans that are addressed by other relevant international agreements or organizations (Article 5)

The Protocol does "*not apply to the transboundary movement of living modified organisms which are pharmaceuticals for humans that are addressed by other relevant international agreements or organizations*" (Article 5 Cartagena Protocol on Biosafety). Synthetic biology is already being used to produce pharmaceuticals for humans (see section 3). Synthetic biology techniques are anticipated to play a major role in future pharmaceutical development and production (Tan et al., 2021) as is already becoming evident when taking into account synthetic viral vaccines or mRNA-based vaccines for SARS-CoV-2 (Forni & Mantovani, 2021; Rappuoli et al., 2021).

Where synthetic biology organisms are being used as "biofactories" to produce pharmaceuticals, such as in the case of artemisinin, the organisms themselves are not pharmaceuticals. These organisms therefore are not eligible for exemption under Article 5 (see Mackenzie et al., 2003). Vaccines produced using synthetic biology techniques, however, would likely be considered pharmaceuticals under Article 5 of the Protocol.[106] Future advances in synthetic biology, such as gene therapy through artificial chromosomes and modifying bacteria and viruses to identify malignant cells and deliver therapeutic agents, may be considered pharmaceuticals.

LMOs that are pharmaceuticals for humans must also be addressed by other relevant international agreements or organizations to be exempted from the Protocol. It is unclear to what extent LMOs that are pharmaceuticals for humans would need to be "addressed" by other international agreement or organization to qualify for the Article 5 exemption. For example, the World Health Organization, the International Council for Harmonization, and many other international bodies address pharmaceuticals for humans, including vaccines and biologics; however, it is an open question whether the agreement or organization must address the biodiversity impacts of the LMO (Mackenzie et al., 2003).

8.2.3. Other relevant provisions of the Cartagena Protocol on Biosafety

Other provisions of the Protocol which may potentially be relevant to the regulation of synthetic biology applications include the following:

(a) Unintentional transboundary movements and emergency measures

Article 17 of the Protocol deals with unintentional transboundary movements and emergency measures in such cases. The article requires each Party to the Protocol to take measures to notify affected or potentially affected States, the Biosafety Clearing-House and, where appropriate, international organizations, when it knows of an occurrence under its jurisdiction resulting in a release that leads, or may lead, to an unintentional transboundary movement of an LMO that is likely to have significant adverse effects on biodiversity, taking also into account risks to human health in such States (Article 17(1)). While the obligation is on Parties to the Protocol, the notification requirement pertains to any affected or potentially affected States, whether or not they are Parties to the Protocol.

The types of emergency responses and actions that may be taken in relation to an unintentional transboundary movement are not specified but are to be determined by the States concerned presumably in light of the nature and scale of the transboundary movement in question and the possible adverse effects on biodiversity and human health (Mackenzie et al., 2003). Furthermore, each Party under whose jurisdiction the release of an LMO occurs that leads or may lead to an unintentional transboundary movement as described in paragraph 1 of Article 17 is required to immediately consult the affected or potentially affected States to enable them to determine appropriate responses and initiate necessary action, including emergency measures (Article 17(4)).

106 The IUCN guide to the Cartagena Protocol reported that LMOs that are pharmaceuticals for humans are "principally genetically engineered vaccines"(Mackenzie et al., 2003).

(b) Illegal transboundary movements
Article 25 of the Protocol addresses the situation where transboundary movement of LMOs takes place in contravention of national regulations implementing the Protocol. Such transboundary movements are deemed illegal. In essence, Article 25 requires each Party to adopt domestic measures to prevent and (if appropriate) penalize transboundary movements of LMOs which contravene its national measures to implement the Protocol (Mackenzie et al., 2003). A Party affected by an illegal transboundary movement of LMOs may request the Party of origin to dispose of the LMOs in question at its own expense. Parties are also required to exchange information through the Biosafety Clearing-House on illegal transboundary movements of LMOs.

(c) Importation and socioeconomic considerations
Article 26 of the Protocol enables Parties in their decision-making to take into account, consistent with their international obligations, socioeconomic considerations arising from the impact of LMOs on the conservation and sustainable use of biodiversity, especially with regard to the value of biological diversity to indigenous and local communities. Additionally, Article 26 encourages Parties to cooperate on research and information exchange on any socioeconomic impacts of LMOs, especially on indigenous and local communities.

(d) Capacity-building
Article 22 of the Protocol commits the Parties to cooperate in the development and/or strengthening of human resources and institutional capacities in biosafety for the purpose of effective implementation of the Protocol in developing country Parties. The article emphasizes the need for capacity-building in least developed and small island developing States. Cooperation in capacity-building is closely linked to the provisions of the Convention on Biological Diversity related to technology transfer as well as scientific and technical development which are discussed in subsection 8.1.6.

(e) Handling, transport, packaging and identification
Article 18 of the Protocol requires Parties to take measures for the safe handling, packaging and transport of LMOs that undergo intentional transboundary movement in order to minimize risks to biodiversity and human health. This applies to all LMOs within the scope of the Cartagena Protocol on Biosafety, whether or not they are subject to the AIA procedure described in subsection 8.2.2(b) (Mackenzie et al., 2003). The Article sets out what information must be provided in the documentation accompanying transboundary movement of LMOs in order to facilitate identification and tracking. Specific requirements vary according to the intended use, with different notification procedures applying to LMOs intended for direct use as food or feed, or processing; LMOs destined for contained use in the Party of import; and LMOs intended for introduction into the environment of the Party of import.

(f) Public awareness and participation
Article 23 of the Protocol provides for a mix of mandatory and discretionary actions that Parties to the Protocol are expected to undertake relating to the provision of information on LMOs to the public, public participation in LMO-related decision-making processes, and the provision of information to the public about access to the Biosafety Clearing-House (Mackenzie et al., 2003). These actions are best understood in the context of Principle 10 of the 1992 Rio Declaration on Environment and Development which articulates the "three pillars" of public participation: (1) the right of citizens to information; (2) their right to participate in environmental decisions which affect them; and (3) their access to mechanisms of redress and justice when their rights are violated.

8.3. Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety

The issue of liability and redress for damage resulting from the transboundary movements of LMOs was one of the themes on the agenda during the negotiation of the Cartagena Protocol on Biosafety. The negotiators were, however, unable to reach any consensus regarding the details of a liability regime under the Protocol and so instead included an article requiring a further process on this issue following

the entry into force of the Cartagena Protocol on Biosafety (Article 27). The result of this process was the Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety (Supplementary Protocol), which was adopted by the Conference of the Parties serving as the meeting to the Parties to the Cartagena Protocol on Biosafety at its fifth meeting, in 2010. The Supplementary Protocol entered into force on 5 March 2018 and had 49 Parties as of March 2022.

The objective of the Supplementary Protocol is to contribute to the conservation and sustainable use of biological diversity, taking also into account risks to human health, by providing international rules and procedures in the field of liability and redress relating to LMOs (Article 1 of the Supplementary Protocol).

The Supplementary Protocol applies to damage resulting from LMOs which finds its origin in a transboundary movement. "Damage" is defined by the Supplementary Protocol (Article 2) as an adverse effect on the conservation and sustainable use of biological diversity, taking also into account risks to human health, that is measurable or otherwise observable taking into account, wherever available, scientifically established baselines recognized by a competent authority that takes into account any other human induced variation and natural variation, and that is significant.

As discussed in subsection 8.2.1 above, organisms resulting from synthetic biology techniques may fall under the definition of a "living modified organism" under the Cartagena Protocol on Biosafety. Further, as described in section 4 above, it is possible that LMOs resulting from synthetic biology techniques could cause adverse effects on the conservation and sustainable use of biological diversity. Views vary quite widely on the scope and therefore "significance" of the potential damage that could be caused by LMOs resulting from synthetic biology techniques (see section 4.1 and subsection 6.1.1). The implications, in terms of determinations of "damage" according to the provisions of the Supplementary Protocol, and its measurability and significance, would need to be considered on a case-by-case basis.

8.4. Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity (the Nagoya Protocol) was adopted on 29 October 2010 and entered into force on 12 October 2014. It had 132 Parties as of March 2022.[107]

The Nagoya Protocol aims to support the implementation of the third objective of the Convention and builds on its provisions, including Article 15, by setting out core obligations for Parties in relation to access to genetic resources and traditional knowledge associated with genetic resources, benefit-sharing and compliance.

The following examines issues relevant to the application of the Nagoya Protocol to uses of synthetic biology.

8.4.1. Synthetic biology and the "utilization of genetic resources"

Article 2 of the Nagoya Protocol addresses the use of terms. It provides that the terms defined in Article 2 of the Convention also apply to the Nagoya Protocol; consequently, the discussions on the definitions of "genetic resources" and "genetic material" in subsection 8.1.5 above are also relevant for the present section. Article 2 of the Nagoya Protocol defines "utilization of genetic resources" as the conducting of research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology. Furthermore, "biotechnology" as defined in Article 2 of both the Convention and the Nagoya Protocol means any technological application that uses

107 See http://www.cbd.int/abs/nagoya-protocol/signatories/default.shtml.

biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use. These definitions can help to clarify the scope of access and benefit-sharing obligations.

The Nagoya Protocol also contains a definition of "derivative" as a naturally occurring biochemical compound resulting from the genetic expression or metabolism of biological or genetic resources, even if it does not contain functional units of heredity.

Synthetic biology applications may be a way of "utilizing" genetic resources as defined in the Nagoya Protocol and the definitions can also help to determine which activities related to synthetic biology would be within the scope of the Nagoya Protocol. As noted in section 3.2, a number of synthetic biology applications concerning crops are under development or commercially available in the agricultural sector. If such crops are used *solely* as a feedstock, this would likely not fall within the "utilization of genetic resources". However, if research on the genetic and biochemical composition was conducted on such crops to determine if they were an appropriate feedstock or could be transformed to be more suitable, this research could be "utilization" within the terms of the Nagoya Protocol, and access to the crops for this purpose would be subject to applicable access obligations of the Nagoya Protocol and domestic legislation or regulatory requirements implementing these obligations.

8.4.2. *Benefit-sharing and the degree of modification of genetic resources*

Synthetic biology techniques provide ways to modify naturally occurring genetic resources so that they better serve specific purposes. One method is by directed evolution, such as the multiplex automated genome engineering technology mentioned in section 1.2, which can generate billions of different mutant genomes per day, performing up to 50 different genome alterations at nearly the same time, using synthetic DNA (Wang et al., 2009).

While not unique to synthetic biology, a question that arises is the extent to which a genetic resource continues to be subject to benefit-sharing obligations, particularly where it undergoes multiple (subsequent) applications and modifications. According to Greiber et al. (2012) this is meant to extend benefit-sharing to processes and products developed along the value chain. Article 5, paragraph 1 of the Nagoya Protocol also provides that "*such sharing shall be upon mutually agreed terms*".

National implementation and the negotiation of mutually agreed terms can assist Parties to an access and benefit-sharing agreement to clarify how far along the value chain the obligations to share benefits would continue to apply to components, organisms and products resulting from synthetic biology. Furthermore, as described in subsection 8.1.5, discussions are ongoing under the Convention and the Nagoya Protocol on the issue of benefit-sharing from the use of digital sequence information on genetic resources.

8.4.3. *Derivatives and synthetic biology*[108]

Synthetic biology raises a number of questions in relation to the application of the Nagoya Protocol to derivatives, for instance whether or not biochemical compounds produced by synthesized organisms could be considered a "derivative" as defined by the Nagoya Protocol (see subsection 8.4.1 for the Protocol's definition of "derivative").

For example, a valuable natural derivative is isoprene, the major molecule of rubber. The enzyme isoprene synthase has only been found in plants – namely, *Hevea brasiliensis,* the rubber tree – but plant genes are not efficiently expressed in microorganisms (Erickson et al., 2011). The Genencor Division of Danisco and the Goodyear Tire and Rubber Company have partnered in research to develop "BioIsoprene", using synthetic biology in the "*construction of a gene that encodes the same amino acid sequence as the plant enzyme but is optimized for expression in engineered microorganisms*" (Erickson et al., 2011).

108 It should be noted that this document is made available for the information of Parties to the Convention and is not intended to affect the rights and obligations of Parties to the Convention or its Protocols.

An initial question is whether genetic resources from *H. brasiliensis* were actually accessed and "utilized" in the context of the Nagoya Protocol. A second question concerns the extent to which benefit-sharing obligations apply to derivatives of organisms resulting from synthetic biology techniques, such as isoprene.

There are different interpretations regarding how the Nagoya Protocol applies to derivatives. It could be argued that the benefit-sharing obligations apply to derivatives through linkages with the definitions of utilization of genetic resources and biotechnology (Article 2 of the Nagoya Protocol) (see Greiber et al., 2012; Nijar, 2011). Another possible interpretation is that the operative provisions of the Protocol apply only to genetic resources, and not to derivatives.[109]

National implementation of the Nagoya Protocol can assist in further clarifying the definition of "utilization" as well as the scope of access and benefit-sharing requirements in relation to derivatives. The negotiation of mutually agreed terms can assist parties to access and benefit-sharing agreements to clarify how far along the value chain the obligations to share benefits would continue to apply to components, organisms and products resulting from synthetic biology, including derivatives and their subsequent applications.

9. Other relevant international treaties, laws, processes and initiatives with implications for the governance of synthetic biology

9.1. Overview

At the international level, the governance of synthetic biology will be determined having regard to a number of factors, including the products and processes involved, the purpose for which they are applied, and the cross-border implications of their use. Accordingly, a wide range of international treaties, laws, processes and initiatives in addition to the Convention on Biological Diversity and its Protocols are anticipated to shape the governance of synthetic biology and as a consequence, there can be multiple national laws and regulations and overlapping responsibilities at the national level. Section 9 considers other international treaties, laws, processes and initiatives with potential implications for the governance of synthetic biology which are relevant to the work of the Convention, as summarized in table 3 below.

A focus on protection of people and the environment appears as a common denominator in the analysis of international governance frameworks which are particularly relevant to synthetic biology (Beeckman & Rüdelsheim, 2020). Given this focus, the Convention on Biological Diversity and its Cartagena Protocol on Biosafety tend to feature as the primary lens through which the governance of synthetic biology applications and products are evaluated, with considerable attention on risk assessment and risk management principles associated with biosafety. Evaluation of other international treaties, laws, processes and initiatives tend to focus on governance overlaps associated with biodiversity conservation and use, biosafety and biosecurity, phytosanitary measures associated with plant and animal health in trade, and access and benefit-sharing frameworks associated with access to genetic resources (Beeckman & Rüdelsheim, 2020; Keiper & Atanassova, 2020; Lai et al., 2019; Trump et al., 2020). Limited technical analysis concerning potential gaps and areas of convergence in the international governance of synthetic biology is available in the peer-review literature beyond issues concerning biosafety and risk assessment. Section 9 prioritizes coverage of initiatives or organizations that are engaged in discussions on synthetic biology or which have programmes of work which consider aspects related to synthetic biology. However, as acknowledged in the scope and limitations noted in section A, this section cannot be construed as an exhaustive coverage of international treaties, laws, processes and initiatives which

109 See Nijar (2011) for descriptions of the arguments for differing interpretations of the role of derivatives in the Nagoya Protocol.

have potential implications for the governance of synthetic biology.

Analysis of the provisions of the Convention and its Protocols in section 8, combined with the mapping of the broader international regulatory landscape in section 9, forms the basis for the discussion in section 10 of the potential gaps and overlaps in the international governance of synthetic biology.

9.2. *International treaties, laws, processes and initiatives with a substantive programme of work addressing synthetic biology*

9.2.1. World Health Organization (WHO)

The World Health Organization is a specialized agency of the United Nations responsible for international public health. It has 194 member States and is governed pursuant to the WHO Constitution, which establishes the World Health Assembly as WHO's governing body, which meets annually. Various areas of work within this organization could be related to or have an impact on synthetic biology governance. These areas are described below.

(a) Responsible life sciences research

In 2010, WHO published a guidance document on responsible life sciences research for global health security[110] which reviewed the types of life sciences research that may be of concern, offering several examples, including synthetic re-creation of viral genetic material. The document outlined a range of complementary policy options for managing potential risks, including the following (were not intended to be mutually exclusive): (1) research oversight mechanisms; (2) policies for funding agencies, publishers and editors; (3) laws and regulations; (4) codes of conduct and ethics; and (5) awareness-raising and educational initiatives for scientific communities, policymakers and the public. It also proposed a laboratory biorisk management framework and a self-assessment questionnaire for those undertaking life sciences research that could be misused, including the use of synthetic biology technologies.

In 2020, WHO organized three dialogues on dual-use research of concern, with academies and councils, science editors and publishers, and research donors respectively, to discuss and learn about current activities and challenges in this area. Since the beginning of 2021, WHO has been developing a Global Guidance Framework for the Responsible Use of Life Sciences with a view to updating guidance in this sector, particular in light of advances in the life sciences since 2010. It is expected that the Framework will provide member States and other stakeholders with options to promote the responsible use of the life sciences and to protect against the potential risks caused by accidents and misuse.

(b) International health regulations, pandemic preparedness and biorisk management

In the wake of the COVID-19 pandemic, WHO is undertaking a comprehensive review of the functioning of the International Health Regulations (2005),[111] including provisions for declaring and managing a public health emergency of international concern.

The COVID-19 pandemic has also raised awareness of the need for better prevention of, and response to, possible or evolving public health emergencies of biological origin, whether natural, accidental or deliberate. To this end, WHO and the United Nations Office for Disarmament Affairs (UNODA) have teamed up as co-leads on a United Nations internal system-wide Biorisk Working Group, established by the Secretary-General, which aims at bringing together policy/normative and technical expertise to further develop a clear understanding of capacities, mechanisms, roles and responsibilities, and harmonize them within the United Nations system. This will strengthen the international community's preparedness and response to natural, accidental or deliberate biological events.[112]

110 Responsible Life Sciences Research for Global Health Security. A Guidance Document. Available at https://apps.who.int/iris/handle/10665/70507.

111 https://www.who.int/teams/ihr/ihr-review-committees/covid-19.

112 https://www.un.org/disarmament/unoda-continues-its-mission-on-covid-19/.

Table 3. International treaties, laws, processes, and initiatives with potential implications for the governance of synthetic biology which are relevant to the work of the Convention on Biological Diversity

International treaties, laws processes, and initiatives	Conservation	Sustainable use	Access and benefit-sharing	Other
World Health Organization (WHO)				Health
Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)	x	x		
International Union for Conservation of Nature (IUCN)	x	x		
International customary law related to responsibility and mitigation of harm *(including concerning State responsibility and liability of private actors, prevention of transboundary harm to the environment, environmental impact assessment, and the precautionary approach)*				Risk (general, including health and environment)
Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (Biological Weapons Convention - BWC)				Risk (health; food security)
Convention on the Prohibition of Military or Any Other Hostile Use of Environmental Modification Techniques (Environmental Modification Convention - ENMOD)				Risk (environment)
United Nations Declaration on the Rights of Indigenous Peoples (UNDRIP)	x	x	x	
World Intellectual Property Organization (WIPO) Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC)	x	x	x	
International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)	x	x	x	Food security
International legally binding instrument under the United Nations Convention on the Law of the Sea (UNCLOS) on the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction, under development	x	x	x	Marine genetic resources in areas beyond national jurisdiction
World Trade Organization (WTO) Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS)		x		Intellectual property
International Convention for the Protection of New Varieties of Plants (UPOV Convention)		x		Intellectual property
Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)		x		Trade; risk (phytosanitary)
International Plant Protection Convention (IPPC)		x		Trade; risk (phytosanitary)
World Organisation for Animal Health (OIE)		x		Trade
Codex Alimentarius		x		Trade

(c) Synthetic biology in relation to smallpox preparedness and control

At the Sixty-seventh World Health Assembly, in May 2014, WHO was requested to undertake a consultation on the use and potential impact of technologies for synthetic biology on smallpox preparedness and control, in order to further inform the World Health Assembly in its discussions on the timing of the destruction of existing variola virus stocks. As part of this consultative process a group of experts, the Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox, was convened at the end of June 2015. Additionally, a Scientific Working Group (SWG) Meeting on Synthetic Biology and Variola Virus and Smallpox was convened in April 2015 to provide the technical and scientific background for the Independent Advisory Group.

As noted in the Independent Advisory Group's report (WHO, 2015) it was concluded that the risk of the re-emergence of smallpox overall had increased. They recognized that the creation of the variola virus, using information on DNA sequences, would be easier and cheaper in the future, and may be possible including in small laboratories that have inadequate biosafety and biosecurity for handling the virus. Following these synthetic biology consultations, WHO sought input from its Advisory Committee on Variola Virus Research, and in 2016 updated its recommendations concerning the distribution, handling and synthesis of variola virus DNA.[113] These recommendations are currently under review in order to address the emerging issue of research involving variola virus DNA that may be present in human remains or museum specimens.

(d) Synthetic biology in relation to genetically modified mosquitoes for the control of vector-borne diseases

In October 2020, WHO issued a position statement to clarify its stance on the evaluation and use of genetically modified mosquitoes (GMMs) for the control of vector-borne diseases (VBDs) (WHO, 2020).[114] The statement was issued in accordance with its mandate to provide guidance to member States on health policy and in response to enquiries from member States and their implementing partners about the Organization's position on both research on and deployment of GMMs to reduce or prevent transmission of VBDs.

The main elements of WHO's position are summarized in the position statement as follows:

- "*VBDs cause more than 700 000 deaths annually and are responsible for 17% of the global burden of communicable diseases. Significant progress was made in the control of malaria until 2015, but progress has stalled in recent years. WHO recognizes the urgent need for development and testing of new tools to combat VBDs and supports investigation of all new potential control technologies, including GMMs.*

- *In order to maintain the gains made so far and to advance further towards the elimination and eventual eradication of VBDs, the development and testing of new tools to control both the pathogens and the vectors are urgently needed. WHO actively encourages innovation in this field.*

- *New technologies, including GMMs, may supplement or provide alternatives to existing interventions and may further reduce or even prevent disease transmission. Computer simulation modelling indicates that GMMs could be a valuable new tool in efforts to eliminate malaria and to control Aedes-borne VBDs. Use of GMMs, however, raises concerns about ethics, safety and governance and questions of affordability and cost–effectiveness which must be addressed. In the spirit of fostering innovation, WHO takes the position that all potentially beneficial new technologies, including GMMs, should be investigated to determine whether they could be useful in the continued fight against diseases of public health concern. Such research*

113 WHO Recommendations concerning the distribution, handling and synthesis of Variola virus DNA (2016), available at https://www.who.int/publications/i/item/10665-241232.

114 WHO. Evaluation of genetically modified mosquitoes for the control of vector-borne diseases. Position statement. https://www.who.int/publications/i/item/9789240013155.

should be conducted in steps and be supported by clear governance mechanisms to evaluate the health, environmental and ecological implications.

- *Current mechanisms of governance and oversight, from global to national and institutional levels, must be adapted to the purpose rather than replaced. Existing governance mechanisms should be backed financially to ensure that they are effective.*

- *Internationally recognized risk assessment tools and procedures should be used for evaluating safety. Decisions on evaluation of GMMs should account for the potential benefits to health in terms of disease control and not be limited to potential environmental risk.*

- *Community engagement is essential in developing effective approaches to combating VBDs. Communities must be engaged in planning and conducting field trials before any new public health intervention is introduced. WHO considers that tools for engaging populations affected by VBDs are a priority in field research on GMMs.*"

WHO's position statement includes a recommendation addressing the testing of GMMs as follows:

> "*WHO recommends a stepwise approach to testing GMMs. Oversight mechanisms established by WHO for new vector control interventions are relevant, in addition to those established under the Convention on Biological Diversity; national and institutional mechanisms are also applicable. New vector control interventions should be evaluated with internationally recognized procedures for risk assessment, with account taken of potential health benefit. Substantive engagement of communities, including under-represented and indigenous populations, is a priority in field trials of any new VBD control strategy and of any new public health intervention strategy.*"

Additionally, on behalf of the Special Programme for Research and Training in Tropical Diseases, in May 2021, WHO published a second edition of its *Guidance Framework for Testing Genetically Modified Mosquitoes*. This revised the guidance framework first published in 2014 and provides best practice recommendations to facilitate decision-making by countries interested in the potential use of GMMs as public health tools for the control of VBDs. It also provides detailed guidance on efficacy and safety evaluations, as well as ethical and regulatory considerations, taking into account the technical progress made and lessons learned in the rapidly advancing field, including developments in relation to gene drives (WHO, 2014; WHO, 2021).

(e) WHO Laboratory Biosafety Manual[115]

WHO released a fourth edition of its WHO Laboratory Biosafety Manual in December 2020 (WHO, 2020). The manual has been in broad use at all levels of clinical and public health laboratories, and other biomedical sectors globally, serving as a *de facto* global standard that presents best practices and sets trends in biosafety in a laboratory (i.e. contained use) setting. The Laboratory Biosafety Manual encourages countries to accept and implement basic concepts in biological safety and to develop national codes of practice for the safe handling of biological agents in laboratories within their geographical borders.

This fourth edition of the manual builds on the risk assessment framework introduced in the third edition. WHO asserts that an evidence-based and transparent assessment of the risks allows safety measures to be balanced with the actual risk of working with biological agents on a case-by-case basis, and that this novel evidence- and risk-based approach will allow optimized resource use and sustainable laboratory biosafety and biosecurity policies and practices that are relevant to their individual circumstances and priorities, enabling equitable access to clinical and public health laboratory tests and biomedical research opportunities without compromising safety.

115 Laboratory biosafety manual, 4th edition: core document. Available at https://www.who.int/publications/i/item/9789240011311.

Synthetic biology is recognized in section 8.8 of the manual as an emerging technology (alongside genetically modified microorganisms, gain-of-function research, stem cell research, genome editing and gene drives) which, if conducted responsibly, safely and securely, can improve global health security and contribute to economic development, evidence-informed policymaking, and public trust and confidence in science. However, countries, laboratories and scientists are cautioned to also consider the risks posed by incidents and/or the potential deliberate misuse of life sciences research and to select appropriate control measures to minimize those risks in order to conduct necessary and beneficial life sciences research. Further, WHO also advises to not focus on any one of these emerging technologies but rather use one framework in which risks can be assessed and managed regardless of the technology involved.

(f) Pandemic Influenza Preparedness (PIP) Framework
WHO's PIP Framework was unanimously adopted by the Sixty-fourth World Health Assembly in 2011.[116] The PIP Framework brings together member States, industry, other stakeholders and WHO to implement a global approach to pandemic influenza preparedness and response. Its key goals include improving and strengthening the sharing of influenza viruses with human pandemic potential through the Global Influenza Surveillance and Response System (GISRS), an international network of influenza laboratories that conduct year-round surveillance of influenza,[117] and increasing the access of developing countries to vaccines and other pandemic related supplies.

WHO is currently implementing the PIP Partnership Contribution High-Level Implementation Plan II (2018-2023), which includes the following key features, as described by WHO:

- Partnership contribution – An annual cash contribution of US$ 28 million is given to the WHO by influenza vaccine, diagnostic and pharmaceutical manufacturers that use the WHO Global Influenza Surveillance and Response System. This money is used to prepare for – and respond to – an influenza pandemic.

- The Standard Material Transfer Agreement 2 is an advance supply contract that will give the WHO predictable access to vaccines and other products needed during the response to the next influenza pandemic. The WHO signs these contracts with manufacturers, research institutions, or other entities that receive PIP biological materials (PIPBM) – or, in some cases, benefit from the use of PIPBM – from a laboratory which is part of the GISRS.

- Virus sharing – Influenza virus sharing conducted by the GISRS is vital to global pandemic preparedness. The sharing of viruses facilitates pandemic risk assessment, the development of candidate vaccine viruses, updating of diagnostic reagents and test kits, and surveillance for resistance to antiviral medicines.

- Influenza virus traceability – The Influenza Virus Traceability Mechanism (IVTM) is a publicly accessible, electronic, internet-based system that records the transfer and movement of PIP biological materials into, within and to parties outside the WHO GISRS. The purpose of the system is to allow users to see where PIP biological materials have been sent.

- The IVTM increases the transparency of GISRS activities by allowing users to track the transfers of PIP biological materials. It enables users to see the results of analyses and tests carried out with them.

The PIP Framework contains provisions related to digital sequence information. It refers to "genetic sequences", which is defined to mean "*the order of nucleotides found in a molecule of DNA or RNA. They contain the genetic information that determines the biological characteristics of an organism or virus*".

116 https://apps.who.int/gb/pip/pdf_files/pandemic-influenza-preparedness-en.pdf.
117 https://www.who.int/initiatives/global-influenza-surveillance-and-response-system/virus-sharing.

Section 5.2 of the PIP Framework addresses genetic sequence data and paragraph 1 provides that "*genetic sequence data, and analyses arising from that data, relating to H5N1 and other influenza viruses with human pandemic potential should be shared in a rapid, timely and systematic manner with the originating laboratory and among WHO GISRS laboratories*".

Furthermore, in both Annex 4 and Annex 5 to the PIP Framework, WHO global influenza surveillance and response system laboratories are required to "*submit genetic sequences data to GISAID and Genbank or similar databases in a timely manner consistent with the Standard Material Transfer Agreement*" (para. 9).

The PIP Framework is the only pathogen-specific international access and benefit-sharing (ABS) instrument. Similarly to ITPGRFA, it uses a standard material transfer agreement to implement a multilateral system for access and benefit-sharing; however, the design of the benefit-sharing arrangements in the two systems is substantially different (see subsection 9.3.3(a) regarding ITPGRFA).

(g) One Health

Recognizing that the health of people is closely connected to the health of animals and our shared environment, WHO is promoting a "One Health" multidisciplinary approach[118] to the designing and implementing of programmes, policies, legislation and research which requires the input and coordination of multiple sectors to communicate and work together in order to achieve better public and animal health outcomes. This is particularly relevant for food safety, the control of zoonoses, and combating antibiotic resistance, when the sharing of epidemiological data and laboratory information can help to effectively detect, respond to and prevent outbreaks of such problems. The "One Health" approach involves a number of international institutions, especially WHO, FAO, OIE and the Convention, to promote comprehensive responses to public, livestock and environmental health threats and to provide guidance on how to reduce these risks. Effective implementation is heavily reliant upon the active collaborative efforts of many government officials, researchers and experts such as epidemiologists, physicians, and veterinarians at the local, national, regional and global levels.

9.2.2. Convention on International Trade in Endangered Species of Wild Fauna and Flora

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international treaty which provides a framework for Parties to adopt domestic legislation to ensure that international trade in specimens of wild animals and plants does not threaten their survival. CITES currently has 184 Parties (as of March 2022) and accords varying degrees of protection to more than 35,000 species of animals and plants.

In 2016, CITES commenced a programme of work focused on "*specimens produced from synthetic or cultured DNA*" and this has recently undergone a change in terminology to focus on "*specimens produced through biotechnology*". At its seventeenth meeting, in 2016, the Conference of the Parties to CITES requested the CITES secretariat[119] to undertake a review of relevant CITES provisions, resolutions and decisions as they relate to specimens produced from synthetic or cultured DNA, in order to examine:

- How Parties have applied the interpretation of resolutions concerning trade in readily recognizable parts and derivatives, to wildlife products produced from synthetic or cultured DNA;[120]

- Under what circumstances wildlife products produced from synthetic or cultured DNA meet the current interpretation of such resolutions; and

118 https://www.who.int/news-room/q-a-detail/one-health.

119 Pursuant to CITES decisions 17.89 to 17.91, available at https://cites.org/sites/default/files/eng/com/sc/69/E-SC69-35.pdf.

120 Specifically pursuant to Resolution Conf. 9.6 (Rev. CoP16), concerning trade in readily recognizable parts and derivatives, available at https://cites.org/sites/default/files/document/E-Res-09-06-R16_0.pdf.

- Whether any revisions should be considered, with a view to ensuring that such trade does not pose a threat to the survival of CITES-listed species.

- The CITES secretariat commissioned a study on wildlife products produced from synthetic or cultured DNA[121] and requested information from Parties on cases where they have issued (or not issued) CITES permits and certificates for bioengineered specimens, to which one Party reported having issued permits deemed to be products of bioengineering.[122] At its sixty-ninth meeting, in 2017, the Standing Committee of CITES established an intersessional working group on synthetic or cultured DNA with a mandate to review the secretariat's findings and recommendations including, *inter alia*, as follows:

- Although only a few applications are commercially available or known today, biotechnologies, combined with other technological tools such as three-dimensional printing, would allow vast possibilities for making synthetic specimens of almost any CITES-listed species that closely mimic both the physical appearance and biological characteristics of their wildlife counterparts;

- The technologies are evolving constantly, and may pose an increasingly complex landscape to identify, let alone regulate, considering that some will be extremely difficult to differentiate by visual or analytical means;

- In cases where they are indistinguishable, all specimens are suggested to be regulated as if they were from the wild. Even in cases where they can be differentiated, some form of regulation may be necessary.

Should a need arise to create exemptions or simplified procedures to demonstrate that the specimen was produced through biotechnology, the study suggests a number of options may be used to make them "readily recognizable", for which there are a number of possible means. However, the study does not make any conclusive remark on which options should be suitable, or precisely what should be regulated, and how. At its seventieth meeting, in 2018, the Standing Committee of CITES noted the urgency of addressing the issue of synthetic or cultured DNA as driven by the rapid development of the technologies involved, including the concern that rhino horns produced through biotechnology are, or could be, available imminently which are genetically similar or identical to real rhinoceros horn. However, it also noted caution in providing recommendations prematurely. The meeting record indicates some Committee members and Parties expressed diverging views as to whether or not specimens produced by biotechnology fell under the remit of CITES.[123]

The Standing Committee's recommendations were considered at the eighteenth meeting of the Conference of the Parties to CITES, in 2019, and the decisions regarding "specimens produced through biotechnology"[124] included the following directions:[125]

- Parties were invited to provide information regarding (a) cases where they have issued, or received requests to issue, CITES permits and certificates for specimens produced through biotechnology; (b) other situations when they have applied the interpretation of Resolution Conf. 9.6 (Rev. CoP16) on trade in readily recognizable parts and derivatives to fauna and flora products produced through biotechnology; and (c) technological developments and applications taking place, particularly in their

121 The study is available as Annex 6 to document SC70 Doc. 33, available at https://cites.org/sites/default/files/eng/com/sc/70/E-SC70-33-A6.pdf.

122 As noted in the meeting document CoP18 Doc. 43, "Specimens produced from synthetic or cultured DNA", submitted to the eighteenth meeting of the Conference of the Parties to CITES, held 23 May – 3 June 2019.

123 As per the Summary Record, Seventieth meeting of the Standing Committee, SC70 SR, available at https://cites.org/sites/default/files/eng/com/sc/70/exsum/E-SC70-SR.pdf.

124 Note: the change in terminology from "*specimens produced through synthetic or cultured DNA*" to "*specimens produced through biotechnology*" was made in accordance with to the Standing Committee's recommendation.

125 As per decisions 18.147 - 18.150, "Specimens produced through biotechnology", available at https://cites.org/eng/taxonomy/term/42062.

jurisdiction, that may result in the manufacture of specimens produced through biotechnology that may have impact on the interpretation and implementation of the convention;

- The Animal and Plants Committees of CITES were requested to monitor the most recent scientific and technological advancements and applications that may lead to the synthetic production of specimens of CITES-listed species, and to make recommendations, including appropriate revisions to existing resolutions; and to provide any relevant scientific advice and guidance on matters relevant to international trade in specimens produced through biotechnology;

- The CITES secretariat was requested to coordinate with the Secretariat of the Convention on Biological Diversity, the Food and Agricultural Organization of the United Nations, the International Union for Conservation of Nature and other relevant organizations as appropriate, to keep abreast of the discussions taking place on other fora on issues that may be relevant to specimens produced through biotechnology.

9.2.3. International Union for Conservation of Nature

The International Union for Conservation of Nature (IUCN) is a membership Union, composed of government and civil society organizations, with a focus on nature conservation and sustainable development. It has more than 1,400 member organizations and is supported by more than 17,000 experts. Every four years, a World Conservation Congress consisting of a public Forum and a Members' Assembly takes place. The Congress and in particular its Members' Assembly constitutes the Union's highest decision-making body. The IUCN World Conservation Congress convenes several thousand leaders and decision makers from government, civil society, indigenous peoples, business, and academia, with the goal of discussing and deciding upon the world's most pressing conservation challenges and the solutions that nature offers. An IUCN Council operates as the principal governing body of IUCN between sessions of its World Conservation Congress.

Unlike the Convention on Biological Diversity and its Protocols and most other international initiatives considered in the present document, IUCN is not established by contracting parties pursuant to an international treaty. It is, however, considered an international authority in the field of nature conservation and sustainable use of natural resources and its activities – particularly concerning scientific and knowledge development, data gathering and analysis, research, field projects, policy influencing, and education – contribute to international policy development under the Convention and its Protocols.

In 2016, the IUCN Members' Assembly adopted a resolution calling for an evidence-based assessment of the issues regarding synthetic biology that are relevant to and may have an impact – negative or positive – on the conservation and sustainable use of biological diversity (IUCN, 2016). Specifically, it called for IUCN to:

- Examine the organisms, components and products resulting from synthetic biology techniques and the impacts of their production and use, which may be beneficial or detrimental to the conservation and sustainable use of biological diversity and associated social, economic, cultural and ethical considerations;

- Recommend how IUCN, including its Commissions and members, could approach the topic of synthetic biology and engage in ongoing discussions and deliberations with the synthetic biology community;

- Assess the implications of engineered gene drives and related techniques and their potential impacts on the conservation and sustainable use of biological diversity as well as equitable sharing of benefits arising from genetic resources;

- Develop IUCN guidance on this topic, while refraining from supporting or endorsing research, including field trials, into the use of gene drives for conservation or other purposes until this assessment has been undertaken.

This resulted in a significant effort involving a scientific and policy landscape assessment and the establishment of an IUCN Synthetic Biology and Biodiversity Conservation Task Force (2018) which relied on regional consultation activities (2018-2019) to develop a technical assessment to support policy development and provide guidance on biodiversity conservation in relation to synthetic biology. The technical assessment which was published by IUCN in 2019[126] includes detailed coverage of synthetic biology applications which are directly or indirectly intended for conservation benefit, governance challenges raised by synthetic biology and conservation, and an evaluation of governance frameworks relevant to synthetic biology impacts on biodiversity (Redford et al., 2019).

A series of IUCN principles on synthetic biology and biodiversity conservation were proposed in a Council motion[127] submitted for the 2020 IUCN World Conservation Congress that was initially scheduled to take place in June of 2020, but which was held from 3 to 11 September 2021 due to the COVID-19 pandemic. The motion, subsequently approved by the IUCN World Conservation Congress, consisted of the following elements seeking to establish a process towards the development of an IUCN policy on synthetic biology in relation to nature conservation:[128]

- A request to initiate an inclusive and participatory process to develop the policy to be debated and voted on by the next 2024 Conservation Congress, pursuant to terms of reference for an inclusive process;

- A request to create a working group composed of IUCN members (NGOs, governments and indigenous peoples' organizations) ensuring a balance among genders, regions, perspectives and knowledge systems, pursuant to terms of reference for the establishment of the working group;

- A request to establish a drafting and participatory review process for the working group to undertake the development of the policy, pursuant to terms of reference for the policy development process; and

- A call for stakeholders to remain neutral on all aspects of synthetic biology until the formal adoption of the policy on synthetic biology, remaining cognisant as new understanding develops during the process.

9.3. Other international treaties, laws, processes, and initiatives with potential implications for the governance of synthetic biology which have yet to implement a substantive programme of work

9.3.1. Risk of harm

(a) International customary law related to responsibility and mitigation of harm

International law includes a number of overarching rules and principles that are common legal ground and might apply to all activities related to components, organisms and products resulting from synthetic biology techniques. Treaties only apply to those States that are Party to them. In contrast, customary law applies to States (except for so-called "persistent objectors") regardless of whether they are a Party to, and bound by, a particular treaty. Some aspects of customary law, reviewed here, have a scope that may be relevant to components, organisms and products resulting from synthetic biology techniques. These rules and principles may, in particular, be discussed in the context of addressing

126 The technical assessment "Genetic frontiers for conservation: an assessment of synthetic biology and biodiversity conservation: technical assessment" is available at https://portals.iucn.org/library/node/48408. A synthesis and key messages related to the technical assessment is available at https://portals.iucn.org/library/node/48409.

127 Available at https://www.iucn.org/sites/dev/files/decisions_78th_bureau_meeting_19_august_2019_with_annex_1-2.pdf.

128 Motion #75 "*Towards development of an IUCN policy on synthetic biology in relation to nature conservation*" is available at https://www.iucncongress2020.org/motion/075 and its subsequent approval is available at https://www.iucncongress2020.org/assembly/motions.

potential negative effects from synthetic biology techniques. It will not be possible to draw specific conclusions on the extent to which these rules and principles will apply and have consequences for specific synthetic biology techniques, as this depends on the particularities of each specific case. It should be noted that the status of some concepts as *legal* principles or rules is disputed or their precise meaning is unclear.

State responsibility and liability of private actors

State responsibility describes the rules governing the general conditions under which a State is responsible for wrongful actions or omissions, and the resulting legal consequences. The rules on State responsibility presuppose a breach of an international obligation by a State. However, the rules on State responsibility do not define the requirements of the obligation which is said to have been breached. Instead, they deal with the consequences of such a breach.

The rules on State responsibility were codified and developed by the International Law Commission's Articles on Responsibility of States for Internationally Wrongful Acts (see Annex to UNGA resolution 56/83 of 12 December 2001, document A/RES/56/83) (henceforth "Articles on State Responsibility"), which for the most part reflect customary law.[129]

The rules on State responsibility do not define obligations relating to synthetic biology in the sense of determining which activities are permitted or prohibited. Instead, in the absence of specific rules, the rules on State responsibility provide a basic legal framework for activities related to synthetic biology in case they breach other existing international obligations.[130]

State responsibility does not as such require fault or negligence of the State. The conduct required or prohibited and the standards to be observed depend on the specific obligation in question. The consequences of State responsibility include legal obligations to cease the activity, to offer appropriate assurances and guarantees of non-repetition, if circumstances so require, and to make full reparation for the injury caused (Articles 30 and 31 of the Articles on State Responsibility).

The existence of "circumstances precluding wrongfulness", such as self-defence or force majeure (Chapter V of the Articles on State Responsibility), may preclude international responsibility notwithstanding a breach of an international obligation. One of these recognized circumstances is necessity. Article 25 reflects that "*necessity may not be invoked by a State … unless the act is the only way for the State to safeguard an essential interest against a grave and imminent peril*" and "*does not seriously impair the essential interest of the State or States toward which the obligation exists, or to the international community as a whole*". It further provides that "*necessity may not be invoked by a State as a ground for precluding wrongfulness if … the State has contributed to the situation of necessity*" (Article 25 of the Articles on State Responsibility). This may be relevant if synthetic biology techniques, as anticipated, are used to design and construct organisms with environmental functions such as bioremediation and pollution control (see section 3). However, the fact-specific nature of circumstances precluding wrongfulness and their limitation to situations virtually beyond the control of a State limits their utility as an *ex-ante* legal justification.

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129 The rules relevant to the present document are customary law, although some other concepts in the Articles on State Responsibility may not be universally accepted. Previous drafts of the Articles on State Responsibility had introduced the concept of "international crimes", which included serious breaches of certain environmental obligations. However, that concept was subsequently dropped and does not appear in the final outcome of the ILC's work.

130 In addition, and as a result of a separate stream of work, the International Law Commission has also drafted a separate set of articles regarding harmful effects of "hazardous" acts, even where such acts are not in breach of an international obligation, although such principles only refer to the allocation of loss, see for instance the work of the ILC on *Draft Articles on Prevention of Transboundary Harm from Hazardous Activities*, UN Doc. A/56/10. This could include making private actors liable under domestic law, cf. ILC, *Draft principles on the allocation of loss in the case of transboundary harm arising out of hazardous activities,* UN Doc. A/61/10, paragraph 66, in particular principle 4.2 (see also UN Doc. A/66/10). In contrast to many of the Articles on State Responsibility, these draft articles do not reflect customary law.

Synthetic biology techniques may be conducted by both State-governed and private entities. The customary international law on State responsibility, as reflected by the Articles on State Responsibility, addresses the circumstances under which the conduct of non-State actors may be attributable to a State. In general, the conduct of non-State actors is not attributable to a State unless a sufficient nexus in the relationship is present (e.g. a private actor exercising elements of governmental authority).[131] Separately, a primary legal obligation (e.g. a treaty) may obligate a State to ensure the activities of its nationals conform to a certain standard, as in the example of Article 139 of the United Nations Convention on the Law of the Sea. A State could be in breach of an obligation if it fails to take necessary measures to prevent effects caused by private actors. It depends on the obligation in question to what extent a State has to address private actors in order to fulfil its own obligation.

In addition, a State can be under an explicit and specific obligation to address private actors. Specifically, international law can impose a duty on States to provide in their internal law that non-State actors are liable for certain acts. For instance, the 2010 Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety requires States to address private actors through domestic rules on liability. However, there is no general obligation on States to do this.

Prevention of transboundary harm to the environment

The International Court of Justice (ICJ), in the *Gabcikovo-Nagymaros* case, and in its advisory opinion on the *Legality of the Threat or Use of Nuclear Weapons*, confirmed the "*existence of the general obligation of States to ensure that activities within their jurisdiction and control respect the environment of other States or of areas beyond national control is now part of the corpus of international law relating to the environment*".[132] In the *Pulp Mills* case, the Court used a slightly different wording:[133] "*It is 'every State's obligation not to allow knowingly its territory to be used for acts contrary to the rights of other States' (Corfu Channel (United Kingdom v. Albania), Merits, Judgment, I.C.J. Reports 1949, p. 22). A State is thus obliged to use all the means at its disposal in order to avoid activities which take place in its territory, or in any area under its jurisdiction, causing significant damage to the environment of another State*". The Court further clarified that "*the principle of prevention, as a customary rule, has its origins in the due diligence that is required of a State in its territory*".[134]

Article 3 of the Convention on Biological Diversity, entitled "Principle", provides that "*States have, in accordance with the Charter of the United Nations and the principles of international law, the sovereign right to exploit their own resources pursuant to their own environmental policies, and the responsibility to ensure that activities within their jurisdiction or control do not cause damage to the environment of other States or of areas beyond the limits of national jurisdiction*". Principle 2 of the Rio Declaration contains similar language.[135]

The duty not to cause transboundary harm does not mean that any environmental harm, pollution, degradation or impact is for that reason generally prohibited (Birnie et al., 2009). Considering the differences in wording used when referring to the duty not to cause transboundary harm, the precise content of this duty has not been defined. From

131 As per the relationships outlined in the Draft Articles on Prevention of Transboundary Harm from Hazardous Activities.

132 ICJ, Case concerning the Gabcikovo-Nagymaros Project (Hungary v. Slovakia), ICJ Reports 1997, 7, paragraph 53; and Legality of the Threat or Use of Nuclear Weapons (Advisory Opinion - General Assembly), ICJ Reports 1996, 22, paragraph 29.

133 The earliest version of this concept can be found in the Trail Smelter Arbitration, where the arbitral tribunal stated that "*under principles of international law (…) no State has the right to use or permit of its territory in such a manner as to cause injury by fumes on or in the territory of another or the properties therein, if the case is of serious consequence and the injury is established by clear and convincing evidence*", see Trail Smelter Arbitration (United States v. Canada, Reports of International Arbitral Awards, vol.3, 1938 (1941), p. 1965).

134 ICJ, Case concerning Pulp Mills on the River Uruguay (Argentina v. Uruguay), ICJ Reports 2010, 14, paragraph 101.

135 31 ILM 876 (1992); cf. principle 21 of the preceding 1972 Declaration of the United Nations Conference on the Human Environment (Stockholm Declaration), 11 ILM 1416 (1972).

the wording used by the ICJ in the *Pulp Mills* case, it appears that an alleged breach of the duty to not harm the environment, establishing responsibility of a State for an activity related to synthetic biology would require the following elements:

- Significant damage to the environment of another State;
- Activity caused by the State in question/lack of due diligence;
- No circumstances precluding wrongfulness (see the comments above concerning State responsibility and liability of private actors).

Many synthetic biology research and commercial applications have the potential for transboundary environmental impacts. For example, as considered in section 4.1 above, depending on the engineered gene drive system, theoretically, a genetic modification could spread through target populations (non-localized) and persist indefinitely (self-sustaining), or be restricted in spread (localized) or persistence (self-limiting). In practice, impacts on the transboundary environment, if any, would depend on the specific application of synthetic biology. Currently, intentional environmental release of organisms resulting from synthetic biology techniques is limited to a few instances as per the examples noted in section 3.[136] Anticipated applications of synthetic biology include the production of microorganisms specifically designed for environmental release, such as for bioremediation of ocean oil spills (see subsection 3.1.3 concerning synthetic biology applications designed for environmental application in wild settings). Potential environmental harm could also arise from, for example, organisms resulting from synthetic biology techniques that displace existing species because of engineered fitness advantages and become invasive (Abdullah et al., 2019; Redford et al., 2013) or cause populations of non-target invasive species to emerge and increase due to reduced competition or predation following control or eradication of the target species (Sofaer et al., 2018).

While the wording of Article 3 of the Convention on Biological Diversity requires "damage", the wording of the ICJ in the *Pulp Mills* case requires "significant damage". For both cases it is not clear what degree of environmental harm would constitute such damage; however, the definitions of the Supplementary Protocol (refer to section 8.3) provide some guidance in the context of LMOs. "Significant" could be understood to establish a *de minimis* threshold and to require a certain intensity of damage, which appears to be more than just any damage. Whether damage caused by synthetic biology techniques is "significant" would have to be established for the particular case in question.[137]

While the ICJ did not elaborate on the specific requirements for causality, a potential claimant State may have to establish a causal link between the particular synthetic biology activity and, for example, the displacement of a certain species.[138]

In the *Pulp Mills* case, the ICJ also appears to require an element of due diligence, providing for a prohibitive function of the duty not to cause transboundary harm.[139] According to this view, the concept obliges every State of origin to take adequate measures to control and regulate in advance sources of potential significant transboundary harm (Beyerlin & Marauhn, 2011). It is, however, not clear from this case which measures States are required to take in order to prevent such harm. Generally, a State will

136 See also subsection 4.2.5. of the information document UNEP/CBD/COP/12/INF/11 prepared by the Executive Secretary and submitted to the twelfth meeting of the Conference of the Parties.

137 The Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety provides, in its Article 4, a list of factors as basis for determining whether a particular damage is "significant", see section 8.3.

138 In the Supplementary Protocol, a causal link is required between the damage and the LMO (Article 4).

139 Note that the exact relationship between the two dimensions of the no harm concept is still subject to a significant degree of unclarity. All sources seem to agree though that the obligation to prevent represents an essential aspect of the obligation not to cause significant harm (Handl, 2007).

not be in breach of the obligation relevant here unless it fails to apply due diligence.[140] What diligence is "due", however, depends on the circumstances of the particular case related to components, organisms and products resulting from synthetic biology techniques.

In sum, the obligation to prevent transboundary harm depends on the particularities of the specific case and is mainly retrospective. International law provides only very limited means to obtain advance provisional measures in order to stop activities that could be in breach of international obligations.[141] Therefore, the duty not to cause transboundary harm may not be a sufficient instrument to address potential negative impacts from synthetic biology techniques, in particular potential impacts of very low probability but very high magnitude.

Duty to undertake an environmental impact assessment

A further general rule which may be considered to address potential negative impacts resulting from synthetic biology techniques is the duty to carry out an environmental impact assessment.

While Article 14 of the Convention on Biological Diversity also addresses environmental impact assessment, the requirement to carry out an environmental impact assessment for industrial activities that may have a significant adverse impact *in a transboundary context* has even become customary international law and applies to States in the absence of treaty obligations. The ICJ has recognized that the accepted practice among States amounted to "*a requirement under general international law to undertake an environmental impact assessment where there is a risk that the proposed industrial activity may have a significant adverse impact in a transboundary context, in particular, on a shared resource*".[142]

As discussed above in relation to the prevention of transboundary harm to the environment, some of the potential applications of synthetic biology could result in transboundary impacts and could in certain cases have the potential to cause significant adverse impacts. The ICJ referred to activities that "may" have a significant adverse impact. However, it does not establish a threshold of probability for "may".

Independently of the required threshold, there appears to be a lack of consensus among different groups, including scientists, academia, industry, civil society and IPLCs, as to how well the potential dangers related to synthetic biology are known and can be assessed. For example, some synthetic biologists and the Biotechnology Industry Organization have argued that the vast majority of synthetic biology research does not present novel risks compared to existing LMOs and applications of biotechnology and that sufficient accumulated knowledge and expertise is available to characterize associated risks (de Lorenzo, 2010; Erickson et al., 2011). Similarly, a Presidential Commission evaluating the regulatory framework for synthetic biology and emerging technologies in the USA concluded that no new regulations for synthetic biology were needed at the time (Presidential Commission for the Study of Bioethical Issues, 2010). Others, however, are much more cautious about the potential unanticipated risks of synthetic biology (ICSWGSB, 2011; Dana et al., 2012; Friends of the Earth et al., 2012; Gronvall, 2018; Snow & Smith, 2012; Tucker & Zilinskas, 2006). For example, scientific advisory bodies of the European Food Safety Authority recently recommended further guidance concerning aspects of

140 Cf. ILC, *Articles on State Responsibility*, UN Doc. A/56/10, para. 77, Chapter III para. 2; ILC, *Draft articles on prevention of transboundary harm from hazardous activities*, UN Doc. A/56/10, paragraph 98, Article 3 paragraph 8.

141 In recent years the ICJ has only granted two applications for provisional measures, in cases involving the imminent execution of prisoners, LaGrand Case (Germany v. United States of America), Provisional Measures, order of 03.03.1999; Avena and Other Mexican Nationals (Mexico *v.* United States of America), order of 05.02.2003. All other applications were rejected; see Armed Activities on the Territory of the Congo (New Application: 2002) (Democratic Republic of the Congo *v.* Rwanda), order of 10.07.2002; Certain Criminal Proceedings in France (Republic of the Congo *v.* France), order of 17.06.2003; Pulp Mills on the River Uruguay (Argentina *v.* Uruguay), orders of 13.07.2006 and 23.01.2007; Questions relating to the Obligation to Prosecute or Extradite (Belgium *v.* Senegal), order of 28.05.2009; Proceedings instituted by the Republic of Costa Rica against the Republic of Nicaragua, press release of 19.11.2010; all available at http://www.icj-cij.org.

142 ICJ, Case concerning Pulp Mills on the River Uruguay (Argentina v. Uruguay), ICJ Reports 2010, paragraphs 204 -206.

risk assessment and risk management associated with genetically modified insects containing engineered gene drives (EFSA GMO Panel, 2020) and microorganisms obtained through synthetic biology (More et al., 2020). In their comment in *Nature,* Dana et al. (2012) call for a minimal investment of US$ 20-30 million in synthetic biology risk research over the next 10 years. Safe Genes, a DARPA programme that aims to develop tools and methodologies intended to control, counter, and reverse the effects of genome editing, including gene drives, is one example of safety research; however, it has been noted that there is room for more such efforts and that to reduce safety concerns in synthetic biology, more prominent support and funding is required for research into improvements in safety (Gronvall, 2018).

Significant adverse impacts that may occur include those that are of low probability and high consequence. In a March 2013 *Science* editorial, Martin Rees, former president of the UK Royal Society, identified synthetic biology as a potential existential threat, albeit in a sci-fi scenario (Rees, 2013). A recent 2020 perspective in *Risk Analysis: an International Journal*, which analysed the extent to which existential risks have been discussed at an international governance level, specifically in documents in the United Nations Digital Library, argued that member nations should urgently advocate for appropriate action at the United Nations to address existential threats, such as artificial intelligence, synthetic biology, geoengineering, and super-volcanic eruption, in analogous fashion to existing attempts to mitigate the threats from nuclear war or near-Earth objects (Boyd & Wilson, 2020).

The ICJ left it to the States to determine the specific content of the impact assessment required. It specified the following details:

- The duty to carry out an environmental impact assessment for industrial activities that may have a significant adverse impact in a transboundary context involves "*having regard to the nature and magnitude of the proposed development and its likely adverse impact on the environment as well as to the need to exercise due diligence in conducting such an assessment*";

- The impact assessment has to be carried out prior to the implementation of the activity;

- Continuous monitoring of the activity's effect on the environment is required.

As a legal rule in customary international law, the duty to carry out an environmental impact assessment for industrial activities that may have a significant adverse impact in a transboundary context is an important development that might require clarification as to its precise implications.

Precautionary approach

Several multilateral environmental treaties and other instruments include precaution, under various labels, such as "precautionary principle", "a precautionary approach", "the precautionary approach" or "precautionary measures". Some States refer to a "precautionary principle", while others consider that formulations of precaution are too varied to be referred to as a "principle". There is no uniform formulation or usage for the precautionary approach, and its legal status in customary international law has not been clearly established, although it has been invoked several times (Beyerlin & Marauhn, 2011).

Under the Convention on Biological Diversity, a precautionary approach has been introduced in the preamble recognizing that "*where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat*". Similarly, the objective of the Cartagena Protocol on Biosafety makes reference to the the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, and language enabling precautionary decision-making is included in Articles 10 and 11 of the Protocol. The Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress also makes reference to the precautionary approach in its preamble. Furthermore, Article 5, paragraph 3, provides that "*where relevant information, including*

available scientific information or information available in the Biosafety Clearing-House, indicates that there is a sufficient likelihood that damage will result if timely response measures are not taken, the operation shall be required to take appropriate response measures so as to avoid such damage".

The decisions of the Conference of the Parties have frequently been based on and stressed the importance of the precautionary approach (see for example decisions II/10, V/8 and IX/20), and multiple decisions on synthetic biology have called for a precautionary approach (see decisions XI/11, XII/24, XIII/17 and 14/19 and summary in table 2 above).

(b) Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (Biological Weapons Convention)

The Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (Biological Weapons Convention – BWC) entered into force in 1975 and currently has 183 Parties. It includes a general-purpose criterion which applies to all scientific and technological developments whose intended use is inconsistent with the objectives of the BWC. By broadly governing the purpose of biological and chemical agents as opposed to specific objects, the prohibitions under the criterion are not limited to a specific list but encompass all biological and chemical weapons. This ensures that these prohibitions remain relevant regardless of future scientific and technological developments, and that they cover even currently unknown agents and toxins that may be employed as weapons. Accordingly, the BWC may apply to the use of components, organisms and products resulting from synthetic biology techniques for hostile purposes or in armed conflict.[143]

Overview of main provisions

The general-purpose criterion of the BWC is contained in its Article I, in which each Party to the convention undertakes never in any circumstance to develop, produce, stockpile or otherwise acquire or retain (a) microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes; or (b) weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict.

Further, where such agents, toxins, weapons, equipment and means of delivery are in the possession or under the jurisdiction and control of a Party, the Party is obliged to destroy or divert them to peaceful purposes not later than nine months after the entry into force of the convention (Article II BWC). Article III prohibits the transfer of agents, toxins, weapons, equipment and means of delivery to any recipient, and Article IV requires each Party to take any necessary measures at the national level to prohibit and prevent the development, production, stockpiling, acquisition or retention of the agents, toxins, weapons, equipment and means of delivery. Other provisions address consultation among Parties (Article V BWC), establish a complaint system (Article VI BWC) and assistance in the case of a violation of obligations under the Convention (Article VII BWC).

Article X of the Biological Weapons Convention requires its Parties to facilitate, and have the right to participate in, the fullest possible exchange of equipment, materials and scientific and technological information for the use of bacteriological (biological) agents and toxins for peaceful purposes. It also states that the Biological Weapons Convention has to be implemented in a manner designed to avoid hampering the economic or technological development of its Parties or international cooperation in the field of peaceful bacteriological (biological) activities, including the international exchange of bacteriological (biological) agents and toxins and equipment for the processing, use or production of bacteriological (biological) agents and toxins for

143 The Australia Group, an informal forum of countries which, through the harmonization of export controls, seeks to ensure that exports do not contribute to the development of chemical or biological weapons, is also relevant in this context, as noted in section 5.4.

peaceful purposes in accordance with the provisions of the convention.

Microbial or other biological agents, or toxins

The described obligations can apply to components, organisms and products resulting from synthetic biology techniques as far as they are microbial or other biological agents, or toxins. This matter has been addressed by a number of Review Conferences under the Biological Weapons Convention.[144]

The Second Review Conference reiterated that the convention "*unequivocally applies to all natural or artificially created microbial or other biological agents or toxins whatever their origin or method of production. Consequently, toxins (both proteinaceous and non-proteinaceous) of a microbial, animal or vegetable nature and their synthetically produced analogues are covered*" (Biological Weapons Convention, 1986).

In 2016, the Eighth Review Conference adopted a final declaration covering the full scope of the convention which reiterated that the convention "*is comprehensive in its scope and that all naturally or artificially created or altered microbial and other biological agents and toxins, as well as their components, regardless of their origin and method of production and whether they affect humans, animals or plants, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes, are unequivocally covered by Article I*"; and further that "*Article I applies to all scientific and technological developments in the life sciences and in other fields of science relevant to the Convention*" (Biological Weapons Convention, 2016). Thus, any of the areas of synthetic biology research and techniques of synthetic biology would be covered if used to produce such agents or toxins.

Prophylactic, protective or other peaceful purposes

The prohibition in Article I of the Biological Weapons Convention to develop, produce, stockpile or otherwise acquire or retain biological agents and toxins is not absolute. It applies only to types and to quantities that have no justification for prophylactic, protective or other peaceful purposes. During the negotiations of the convention, it was clarified that the term "prophylactic" encompasses medical activities, such as diagnosis, therapy, and immunization, whereas the term "protective" covers the development of protective masks and clothing, air and water filtration systems, detection and warning devices, and decontamination equipment, and must not be interpreted as permitting possession of biological agents and toxins for defence, retaliation or deterrence. The term "other peaceful purposes" was not defined during the negotiations but may be understood to include scientific experimentation (Goldblat, 1997).[145] For the use of bacteriological (biological) agents and toxins for the described peaceful purposes, Article X of the Biological Weapons Convention applies – the obligation to facilitate, and the right to participate in, the fullest possible exchange of equipment, materials, and scientific and technological information.

Review of developments in the field of science and technology

Implementation of the convention is subject to a five-yearly Review Conference and is supported by an intersessional programme consisting of annual Meetings of States Parties preceded by annual Meetings of Experts. This includes a standing agenda item on review of developments in the field of science and technology related to the convention.[146] The 2017 Meeting of the States Parties approved the following developments in the field of science and technology for review at the annual Meetings of

144 A Review Conference is a five-yearly conference of States Parties, which, in accordance with Article XII of the convention reviews the operation of the convention and also considers, *inter alia*, new scientific and technological developments relevant to the convention.

145 Useful coverage of the negotiations is also available in Innovation, Dual Use, and Security: Managing the Risks of Emerging Biological and Chemical Technologies. Tucker Jonathan B., ed. MIT Press, 2012. 356 pp. The book presents a "decision framework" for assessing the security risks of emerging technologies and fashioning governance strategies to manage those risks. This framework is applied to fourteen case studies, including synthetic genomics, DNA shuffling and directed evolution.

146 For references to working documents under the Biological Weapons Convention that address synthetic biology, see UNICRI 2011.

Experts in the period 2018-2020 (Biological Weapons Convention, 2017):[147]

- Review of science and technology developments relevant to the convention, including for the enhanced implementation of all articles of the convention as well as the identification of potential benefits and risks of new science and technology developments relevant to the convention, with a particular attention to positive implications;
- Biological risk assessment and management;
- Development of a voluntary model code of conduct for biological scientists and all relevant personnel, and biosecurity education, by drawing on the work already done on this issue in the context of the convention, adaptable to national requirements;
- Genome editing, taking into consideration, as appropriate, the issues identified above (only considered in 2018);
- Any other science and technology developments of relevance to the convention and also to the activities of relevant multilateral organizations such as WHO, OIE, FAO, IPPC and OPCW. The common understanding reached by States Parties on these topics will be reported at the next meeting of States Parties under the convention, at which time the intersessional programme is also anticipated to be updated.[148] The Ninth Review Conference will review the operation of the convention, taking into account, *inter alia*, new scientific and technological developments relevant to the convention. (The scheduling of these meetings is in flux due to the COVID-19 pandemic).

At past Review Conferences, many States Parties submitted proposals in support of enhancing the science and technology review process under the convention; however, States Parties have failed to agree on exactly how to achieve a more effective science and technology review (Revill et al., 2021). To date, few discernible steps towards the development of an oversight framework, guiding principles, or models to inform risk are apparent; however, certain States Parties have urged taking a systematic approach by successively examining relevant advances, possible methods for assessing risks and benefits, and ways in which to manage risks and realize benefits.[149] In preparation for the Ninth Review Conference, several States Parties have indicated their interest in implementing some form of review mechanism (Revill et al., 2021) .

(c) Convention on the Prohibition of Military or Any Other Hostile Use of Environmental Modification Techniques (Environmental Modification Convention)
The Environmental Modification Convention (ENMOD), formally the Convention on the Prohibition of Military or Any Other Hostile Use of Environmental Modification Techniques, is an international treaty prohibiting the military or other hostile use of environmental modification techniques having widespread, long-lasting, or severe effects. The convention bans weather warfare, which is the use of weather modification techniques for the purposes of inducing damage or destruction. It has 78 States Parties. ENMOD contains a mechanism for a conference of States Parties to be held every 5 to 10 years and if a lapse of more than 10 years occurs for steps to be taken to convene a conference provided a minimum threshold number of States respond affirmatively.[150]

A First Review Conference was held in 1984 and a Second Review Conference was held in 1992. Given the lapse of more than 10 years, the Secretary-General of the United Nations initiated a process of soliciting the views of the States parties to convene

147 https://undocs.org/bwc/msp/2018/mx.2/2.
148 https://meetings.unoda.org/meeting/bwc-msp-2020/.
149 For example, see a submission by the US to the 2020 Meeting of Experts (postponed to August 2021) concerning "Approaches to Governance for Scientific and Technological Advances in the Life Sciences Relevant to the Biological and Toxin Weapons Convention", available at https://undocs.org/BWC/MSP/2020/MX.2/WP.1.
150 https://www.un.org/disarmament/enmod/.

a Third Review; however, the minimum threshold does not appear to have been reached.[151]

Despite the limited activity under the convention, it is noteworthy given the overlap with the Convention on Biological Diversity, which would also appear to regulate some forms of weather modification or geoengineering, which could have implications if synthetic biology would be used in climate or weather modification.

9.3.2. Free, prior and informed consent of indigenous peoples and local communities

(a) Indigenous and Tribal Peoples Convention, 1989 (No. 169)

The Indigenous and Tribal Peoples Convention, 1989 (No. 169), also known as ILO Convention 169, is an International Labour Organization convention which as of March 2021 had been ratified by 23 countries. Under the convention, governments are to "*respect the special importance for the cultures and spiritual values of the peoples concerned of their relationship with the lands or territories, or both as applicable, which they occupy or otherwise use...*" (Article 13). A number of the convention's more general provisions require the "participation" and "coordination" (Article 2), the absence of "force or coercion" (Article 3), "freely expressed wishes" (Article 4), and "consultation" and "free participation" (Article 6) in relation to safeguarding the rights of indigenous people (International Labour Organization, 1989).

(b) United Nations Declaration on the Rights of Indigenous Peoples

The United Nations Declaration on the Rights of Indigenous Peoples (UNDRIP) was adopted by the General Assembly on 13 September 2007 (United Nations, 2007). The efforts to draft a specific instrument dealing with the protection of indigenous peoples worldwide date back over several decades, including a Working Group on Indigenous Populations established in 1982 and overseen by the Sub-Commission on the Promotion and Protection of Human Rights, the main subsidiary body of the United Nations Commission on Human Rights.[152]

UNDRIP establishes a universal framework of minimum standards for the survival, dignity, and well-being of the indigenous peoples of the world. It elaborates on existing human rights standards and fundamental freedoms as they apply to the specific situation of indigenous peoples, including in relation to the use, management and conservation of resources pertaining to their lands.

Free, prior and informed consent enables indigenous peoples to exercise their right to self-determination, as established in Article 3 of UNDRIP with the introduction of mechanisms that they be fully informed and be in a position to freely refuse or accept projects and proposals that affect their rights, including their lands, resources and territories. It constitutes three interrelated and cumulative human rights of indigenous peoples: the right to be consulted; the right to participate; and the right to their lands, territories and resources.[153]

Specifically, Article 32 provides that indigenous peoples have the right to determine and develop priorities and strategies for developing or using their lands or territories and other resources. It requires States to consult and cooperate in good faith with the indigenous peoples concerned in order to obtain their free and informed consent prior to the approval of any project affecting their lands or territories and other resources. Additionally, States are required to provide effective mechanisms for just and fair redress and to take appropriate measures to mitigate adverse environmental, economic, social, cultural or spiritual impact. Further, Article 42 provides that the United Nations, including its bodies and specialized agencies, as well as States shall promote respect for

151 https://geneva-s3.unoda.org/static-unoda-site/pages/templates/enmod/UNSG%2BNV%2Bre%2BENMOD.pdf.
152 In 2006, the United Nations Commission on Human Rights was replaced by the United Nations Human Rights Council.
153 Human Rights Council, Free, prior and informed consent: a human rights-based approach. Study of the Expert Mechanism on the Rights of Indigenous Peoples, 10 August 2018, (A/HRC/39/62).

and full application of the provisions of UNDRIP and follow up its effectiveness.

9.3.3. Access and benefit-sharing

(a) International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)

Overview of main provisions

Article 1 of ITPGRFA states its objectives as the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security. Additionally, Article 9 of ITPGRFA recognizes the enormous contribution that the local and indigenous communities and farmers of all regions of the world, particularly those in the centres of origin and crop diversity, have made and will continue to make for the conservation and development of plant genetic resources which constitute the basis of food and agriculture production throughout the world.[154] Article 2 of ITPGRFA defines plant genetic resources for food and agriculture as any genetic material of plant origin of actual or potential value for food and agriculture. "Genetic material" is defined as any material of plant origin, including reproductive and vegetative propagating material, containing functional units of heredity. These definitions are similar to those of the Convention on Biological Diversity noted in subsection 8.1.5. The main difference between the two treaties is that the definitions under ITPGRFA refer only to material of plant origin. However, plant genetic resources are the raw material and indispensable for crop genetic improvement.

As discussed in section 4.2 above, under potential impacts, agricultural applications of synthetic biology are a focus of current research and this includes the production of specialized plant feedstocks for bioenergy purposes. According to the IUCN explanatory guide to ITPGRFA (Moore & Tymowski, 2005), the treaty text is ambiguous as to whether functional units of heredity are in themselves plant genetic resources for food and agriculture (PGRFA) or are components of PGRFA. Thus, if synthetic biology research is based upon DNA sequences of PGRFA, it may be a matter of interpretation whether the research is utilizing PGRFA.

According to Article 5 of ITPGRFA, Parties are required, subject to certain qualifiers, to promote an integrated approach to the exploration, conservation and sustainable use of plant genetic resources for food and agriculture which includes, in particular, the following activities which may be relevant for synthetic biology techniques:

- Promotion of the collection of plant genetic resources for food and agriculture and relevant associated information on those plant genetic resources that are under threat or are of potential use;

- Promotion of *in situ* conservation of wild crop relatives and wild plants for food production, including in protected areas, by supporting, *inter alia*, the efforts of indigenous and local communities;

- Cooperation to promote the development of an efficient and sustainable system of *ex situ* conservation, giving due attention to the need for adequate documentation, characterization, regeneration and evaluation, and promotion of the development and transfer of appropriate technologies for this purpose with a view to improving the sustainable use of plant genetic resources for food and agriculture;

- Monitoring of the maintenance of the viability, degree of variation, and the genetic integrity of collections of plant genetic resources for food and agriculture;

154 ITPGRFA gives governments the responsibility for implementing Farmers' Rights, and lists elements or measures that could be taken to protect, promote and realize these rights, as summarized on the ITPGRFA website at https://www.fao.org/plant-treaty/areas-of-work/farmers-rights/promote/en/. Additionally, an Inventory on Farmers' Rights, published in 2021, provides a collection of lessons learned and best practices for the implementation of Farmers' Rights from countries around the world and is available on the ITPGRFA website at http://www.fao.org/plant-treaty/news/news-detail/en/c/1401662/.

- Taking steps to minimize or, if possible, eliminate threats to plant genetic resources for food and agriculture.

These obligations are relevant for synthetic biology in that they support the availability of a broad resource base upon which synthetic biology techniques can draw.

Multilateral system of access and benefit-sharing
In Article 10, paragraph 2 of ITPGRFA, Parties established a multilateral system to facilitate access to plant genetic resources for food and agriculture, and to share, in a fair and equitable way, the benefits arising from the utilization of these resources, on a complementary and mutually reinforcing basis. The Multilateral System applies to the plant genetic resources for food and agriculture listed in Annex I to the treaty, a pool of 64 food and forage crops, established according to criteria of food security and interdependence. Some of these Annex I crops are the focus of synthetic biology research. One example is the modification of maize to be a more efficient biofuel feedstock. Also, some synthetic biology research is focused on modifying microorganisms to produce substances that would substitute for Annex I crops, such as lauric acids that are currently produced in part from coconuts.

Article 12 of ITPGRFA requires Parties to provide facilitated access to plant genetic resources for food and agriculture to other Parties, including to legal and natural persons under their jurisdiction. This access is to be granted pursuant to a standard Material Transfer Agreement through the Multilateral System under certain conditions, including:

- Access shall be provided solely for the purpose of utilization and conservation for research, breeding and training for food and agriculture, provided that such purpose does not include chemical, pharmaceutical and/or other non-food/feed industrial uses;

- Recipients shall not claim any intellectual property or other rights that limit the facilitated access to the plant genetic resources for food and agriculture, or their genetic parts or components, in the form received from the Multilateral System;

- Access to plant genetic resources for food and agriculture under development, including material being developed by farmers, shall be at the discretion of its developer, during the period of its development;

- Access to plant genetic resources for food and agriculture protected by intellectual and other property rights shall be consistent with relevant international agreements, and with relevant national laws.

Under Article 13 of ITPGRFA the Parties agree that benefits arising from the use, including commercial, of plant genetic resources for food and agriculture under the Multilateral System shall be shared fairly and equitably through the exchange of information, access to and transfer of technology, capacity-building, and the sharing of the benefits arising from commercialization.

The latter is achieved through a requirement in the Material Transfer Agreement that a recipient who commercializes a product that is a plant genetic resource for food and agriculture and that incorporates material accessed from the Multilateral System shall pay to a trust fund, especially established for this purpose, an equitable share of the benefits arising from the commercialization of that product. Such payment is not required when the product is available without restriction to others for further research and breeding, in which case the recipient who commercializes shall be encouraged to make such payment.

While the ITPGRFA Multilateral System applies only to the plant genetic resources for food and agriculture set out in Annex I to the treaty, genetic resources not listed in Annex I and held by the International Agricultural Research Centres and other international institutions that have signed an agreement with ITPGRFA's Governing Body, are to be exchanged under similar terms and conditions as the Multilateral System. It is to be noted

that some countries now apply, on a voluntary basis, ITPGRFA's standard material transfer agreement to plant genetic resources for food and agriculture not listed in Annex I to ITPGRFA, which means that the conditions of the Multilateral System also apply to those crops.

At its fifth session, in 2013, the Governing Body of ITPGRFA established a process to enhance the functioning of the multilateral system of access and benefit-sharing. A number of issues arose through this process, including how digital sequence information should be addressed under the Treaty and whether to expand Annex I to the Treaty to cover food and forage crops or perhaps even all plant genetic resources for food and agriculture. The final outcomes of this process were to be adopted by the Governing Body at its eighth session, in 2019; however, the Governing Body was unable to come to an agreement. At the time of writing, it is unclear whether and how this work may continue.

It is worth noting that in support of this process, in 2017 the ITPGRFA Secretary commissioned a scoping report on potential implications of new synthetic biology and genomic research trajectories on ITPGRFA (see Welch et al., 2017) to explore how current technologies and practices related to the exchange and use of genetic information are relevant for the treaty.

It is also worth noting that the divergence of views among Parties regarding benefit-sharing from the use of digital sequence information on genetic resources under the Convention and its Nagoya Protocol (as noted in subsection 8.1.5) is mirrored under ITPGRFA. The outcome of this ongoing debate on interpretation may have implications for the products derived from natural sequences – and possibly other types of digital sequence information – using synthetic biology in the context of plant genetic resources for food and agriculture.

More broadly with regard to the transfer of technology, Parties committed to providing and/or facilitating access to technologies for the conservation, characterization, evaluation and use of plant genetic resources for food and agriculture. According to the IUCN guide to ITPGRFA (Moore & Tymowski, 2005), technologies for the use of plant genetic resources include both traditional plant breeding techniques and biotechnological methods, such as molecular markers and recombinant DNA technology.

(b) International legally binding instrument under the United Nations Convention on the Law of the Sea (UNCLOS) on the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction, under development

The United Nations Convention on the Law of the Sea (UNCLOS) is an international agreement that resulted from the third United Nations Conference on the Law of the Sea, which took place between 1973 and 1982. It has 168 Parties.

Since 2004, the international community has discussed a number of issues, including possible options and approaches to promote international cooperation and coordination for the conservation and sustainable use of marine biological diversity beyond areas of national jurisdiction. In 2011, the United Nations General Assembly agreed to address a package of issues, namely the conservation and sustainable use of marine biodiversity in areas beyond national jurisdiction, in particular, together and as a whole, marine genetic resources, including questions on the sharing of benefits, measures such as area-based management tools, including marine protected areas, environmental impact assessments, and capacity-building and the transfer of marine technology. In its resolution 69/292 of 19 June 2015, the General Assembly "*stress[ed] the need for the comprehensive global regime to better address the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction*". In resolution 72/249 of 24 December 2017,[155] the General Assembly decided to convene an Intergovernmental Conference, under the auspices of the United Nations, to elaborate the text of an international legally binding instrument

155 https://undocs.org/en/a/res/72/249.

under UNCLOS on the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction, with a view to developing the instrument as soon as possible.

Marine genetic resources, including the sharing of benefits, have been central to the discussions of the Conference, which is addressing the topics identified in the package agreed in 2011. The first session of the Conference was convened in 2018 and the second and third sessions in 2019. At the third session, delegations began text-based negotiations on the basis of a draft text of an agreement developed by the President of the Conference (A/CONF.232/2019/6). The fourth session of the Conference, which was scheduled to be held in August 2021 pursuant to General Assembly resolution 75/239, was further postponed by the General Assembly to the earliest possible available date in 2022, preferably during the first half of the year. It will consider a revised draft text of an agreement (A/CONF.232/2020/3). Part II of the revised draft text is entirely dedicated to marine genetic resources, including questions on the sharing of benefits, and contains provisions on, *inter alia*, access to marine genetic resources of areas beyond national jurisdiction, access to traditional knowledge of indigenous peoples and local communities associated with marine genetic resources of areas beyond national jurisdiction, sharing of benefits, including modalities for such sharing, and monitoring. Discussions are ongoing on these and other aspects, including on whether the text should address digital sequence information, with terminology in relation to the latter also under discussion.

(c) WIPO Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC)

Established in 2000, the WIPO Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC) is a forum where WIPO member States discuss the intellectual property issues that arise in the context of access to genetic resources and benefit-sharing as well as the protection of traditional knowledge and traditional cultural expressions (the terms "traditional cultural expressions" and "expressions of folklore" are used interchangeably in WIPO discussions). Indigenous peoples and local communities have expressed reservations about negative connotations of the word "folklore".[156]

WIPO's background brief on the IGC[157] explains that work within the intellectual property (IP) community on the protection of traditional cultural expressions goes back to the 1960s. The impetus came from a growing sense in developing countries that folklore embodied creativity and was part of the cultural identity of indigenous peoples and local communities. Therefore, it was seen as worthy of IP protection, especially since new technologies were making traditional cultural expressions increasingly vulnerable to exploitation and misuse. Work on the relationship between IP, traditional knowledge and genetic resources is more recent, and stems from concerns regarding the role that IP protection should play in achieving global policy objectives as varied as the conservation of biodiversity, food security, free and fair trade, and development.

The IGC has divided its discussions into three thematic areas: (a) traditional knowledge, in the narrow sense, refers to practical knowledge, including know-how, practices, skills, and innovations; (b) tangible and intangible forms in which traditional knowledge and cultures are expressed, communicated or manifested, such as songs, stories, music, performances, narratives, and others; and (c) genetic resources, where the IGC aims to complement the frameworks for access and benefit-sharing of genetic resources utilization. In a broad sense, traditional knowledge describes intellectual and intangible cultural heritage, practices, and knowledge systems of indigenous peoples and local communities, including the three thematic areas.

The WIPO General Assembly which took place from 30 September to 9 October 2019 renewed the

156 WIPO, *Intellectual Property and Traditional Cultural Expressions/Folklore*, https://www.wipo.int/edocs/pubdocs/en/tk/913/wipo_pub_913.pdf.

157 https://www.wipo.int/publications/en/series/index.jsp?id=144.

mandate of the IGC for 2020-2021, including to expedite its work, with the objective of finalizing an agreement on an international legal instrument(s), without prejudging the nature of outcome(s), relating to intellectual property which will ensure the balanced and effective protection of genetic resources, traditional knowledge and traditional cultural expressions.[158] The ICG's negotiations to date concerning an international legal instrument(s) relating to intellectual property, genetic resources and traditional knowledge associated with genetic resources have addressed patent disclosure requirements associated with genetic resources, pertaining to country of origin information. ICG discussions have also considered the issue of digital sequence information on genetic resources, which has been a contentious issue under the Convention (see subsection 8.1.5(a) above). Any international instrument adopted pursuant to the ICG process will likely have potential implications for the Convention on Biological Diversity, particularly its third objective concerning equitable benefit-sharing as well as the objectives of its Nagoya Protocol. The latest meeting of the IGC was held in August 2021 and recommended that the mandate of the Committee be renewed for the 2022-2023 biennium.

9.4. *Other international treaties, laws, processes and initiatives which are relevant to the objectives of the Convention*

9.4.1. *Intellectual property*

(a) WTO Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS)

The WTO Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) came into effect on 1 January 1995 and is to date the most comprehensive multilateral agreement on intellectual property.

Overview of main provisions

According to Article 7 (Objectives) of the TRIPS Agreement, the protection and enforcement of intellectual property rights should contribute to the promotion of technological innovation and to the transfer and dissemination of technology, to the mutual advantage of producers and users of technological knowledge and in a manner conducive to social and economic welfare, and to a balance of rights and obligations.

The TRIPS Agreement sets out the minimum standards of protection that each WTO member has to provide in their national regimes for the different areas of intellectual property, including copyright and related rights; trademarks; patents; and the protection of new varieties of plants, among others. For each area, the TRIPS Agreement defines the subject matter to be protected, the rights to be conferred and permissible exceptions to those rights, as well as the minimum duration of protection. Patents and protection of plant varieties are most relevant, for the components, organisms and products resulting from synthetic biology techniques, as well as technologies and tools associated with such techniques; however, copyright and trademarks have also been discussed in the literature (Holman, 2011; Torrance, 2010). Least developed country members are currently not obliged to give effect to the substantive standards of the TRIPS Agreement (apart from general non-discrimination principles) until 1 July 2034, or until such a date on which they cease to be a least developed country member.[159]

Patents

While discovery and invention both play an important role in synthetic biology, only inventions are treated as a patentable subject matter under the TRIPS Agreement. Article 27, paragraph 1 of the TRIPS Agreement states that patents shall be available for any inventions, whether products or processes, in all fields of technology, provided that they are new, involve an inventive step and are capable of industrial application. The TRIPS Agreement, however, provides no definition or interpretation of these criteria. Thus, WTO members have considerable leeway in applying them at the national level (UNCTAD & ICTSD, 2004).

158 https://www.wipo.int/export/sites/www/tk/en/igc/pdf/igc_mandate_2020-2021.pdf.
159 https://docs.wto.org/dol2fe/Pages/SS/directdoc.aspx?filename=q:/IP/C/88.pdf&Open=True.

The criterion of "novelty" is generally understood to mean that the invention has a new feature which must not have been disclosed or available to the public prior to the patent application date: the inventor is granted a patent for something new (UNCTAD & ICTSD, 2004). In addition, the invention must not merely be something new, but also involve an "inventive step", representing a sufficient development over prior art. Depending on the standards that WTO members require for this step, this requirement can serve to exclude trivial or routine "inventions" from being patented (UNCTAD & ICTSD, 2004). In this context, according to patent practice in some countries, discoveries of things already existing in nature are deemed unpatentable in their naturally occurring form, on the basis that they are mere discoveries and not inventions as such (UNCTAD & ICTSD, 2004). Thirdly, the invention must be useful and capable of industrial application, which aims at a direct technical result (UNCTAD & ICTSD, 2004).

Enabling technologies and tools, components, organisms, and products resulting from synthetic biology techniques may fulfil the necessary criteria and may be the subject of patents in one or more jurisdictions. While there has been some controversy in the past as to whether, for example, DNA sequences should constitute patentable subject matter, considering that they are derived from natural ("genomic") DNA sequences, novel genes constructed using synthetic biology techniques will more clearly fulfil the criteria (Torrance, 2010).

While patentable inventions may in principle be found in all areas of technology, the TRIPS Agreement permits, but does not require, WTO members to exclude on public policy grounds certain inventions from the scope of patentable subject matter, even when they otherwise meet the substantive and formal conditions for patentability. Paragraph 2 of Article 27 states that WTO members may exclude from patentability inventions, the prevention within their territory of the commercial exploitation of which is necessary to protect *ordre public* or morality, including to protect human, animal or plant life or health or to avoid serious prejudice to the environment, provided that such exclusion is not made merely because the exploitation is prohibited by their law. Components, organisms and products resulting from synthetic biology techniques could therefore potentially be excluded from patentability in the territory of a WTO member, if the prevention of their commercial exploitation in that territory is necessary in order to protect human, animal or plant life or health or to avoid serious prejudice to the environment. WTO jurisprudence has so far not addressed the specific requirements of this exception.

Some synthetic biology technologies may be considered as contrary to *ordre public* or morality in some countries. The *WTO Handbook* gives possible examples of inventions contrary to morality, such as "processes for the cloning of human beings or for modifying the germ line identity of humans". If a WTO member considered it necessary to protect morality by preventing the commercial exploitation of components, organisms and products resulting from synthetic biotechnologies, this, too, would give grounds for their exclusion from patentable subject matter.

Article 27, paragraph 3 of the TRIPS Agreement allows WTO members to exclude from patentability: (a) diagnostic, therapeutic and surgical methods for the treatment of humans or animals; and (b) plants and animals other than microorganisms, and essentially biological processes for the production of plants or animals other than non-biological and microbiological processes. It states, however, that WTO members have to provide for the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof.

A significant focus of synthetic biology research is on medical applications – including diagnosis, therapeutic treatment, and the production of drugs and vaccines. It would appear that medical applications of synthetic biology could be excludable from patentability to the extent that they constitute diagnostic, therapeutic and surgical *methods* for the treatment of humans or animals.

"Plants and animals", which can be excluded from patentability, are understood to include plants as

such (including transgenic plants), plant varieties (including hybrids), plant cells, seeds and other plant materials, as well as animals (including transgenic) and animal races (UNCTAD & ICTSD, 2004). While current applications of synthetic biology are mostly in microorganisms, synthetic biology research in mammalian and other eukaryotic cells is making rapid progress (Annaluru et al., 2014; Lienert et al., 2014; Wieland & Fussenegger, 2012), and the products of such applications could fall under excludable "plants and animals". For microorganisms, including bacteria, fungi, algae, protozoa or viruses, patents need to be available, as far as they are novel, non-obvious and useful in accordance with Article 27, paragraph 1 of the TRIPS Agreement (UNCTAD & ICTSD, 2004).

The possibility of excluding the patentability of "essentially biological processes" does not extend to "non-biological" processes for the production of plants or animals or any process that uses or modifies microorganisms, such as methods based on modern biotechnology like the insertion of genes in a plant (UNCTAD & ICTSD, 2004). Although there is room for interpretation in the exact meaning of "essentially biological processes", the chemical synthesis of DNA sequences seems to fall outside of this.

Thus, it seems possible for select products of synthetic biology techniques to be excluded from patentability through Article 27, paragraph 3 of the TRIPS Agreement.

A significant extent of the impact of intellectual property in the field of synthetic biology concerns not what formal legal standards are in place, but how intellectual property is managed – for instance, whether patents are applied for and how they are licensed. The TRIPS Agreement does not regulate this aspect directly, although it provides scope for action to deal with abusive licensing practices and provides for public policy exceptions to patent rights; hence, within the TRIPS framework, a wide spectrum of approaches to obtaining and managing patents in this area can be discerned as noted in section 7.4.

(b) International Convention for the Protection of New Varieties of Plants (UPOV Convention)

The International Union for the Protection of New Varieties of Plants (UPOV) was established by the International Convention for the Protection of New Varieties of Plants (UPOV Convention). The UPOV Convention came into force in 1968 and was revised in 1972, 1978 and 1991 in order to reflect technological developments in plant breeding and experience acquired with the application of the convention.[160] UPOV has 78 members. The main objective of UPOV is to provide and promote an effective system of plant variety protection with the aim of encouraging the development of new varieties of plants, for the benefit of society.

Overview of main provisions

The UPOV Convention sets forth standards, including national treatment, for the granting of "breeders' rights" as a *sui generis* form of protection for new plant varieties. A plant variety in accordance with Article 1, paragraph (vi) of the convention is defined as a plant grouping within a single botanical taxon of the lowest known rank, which grouping, irrespective of whether the conditions for the grant of a breeder's right are fully met, can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, distinguished from any other plant grouping by the expression of at least one of the said characteristics and considered as a unit with regard to its suitability for being propagated unchanged.

Additional explanatory notes on the definition of variety under the 1991 Act of the UPOV Convention are available (document UPOV/EXN/VAR/1).[161]

Breeder's right

In order to be eligible for protection, a plant variety must meet the following requirements (Article 5 UPOV Convention):

160 Unless otherwise stated, reference to the UPOV Convention in the following refers to the 1991 Act of the UPOV Convention.

161 International Union for the Protection of New Varieties of Plants (2010), https://www.upov.int/edocs/expndocs/en/upov_exn_var.pdf.

- Novelty – propagating or harvested material of the variety must not have been sold or otherwise disposed of to others, by or with the consent of the breeder in the territory of the UPOV member where the applicant seeks protection for more than one year, nor for more than four years in any other territory and six years in the case of vines and trees (Article 6);

- Distinctness – the variety must be clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filing of the application (Article 7);

- Uniformity – subject to the variation that may be expected from the particular features of its propagation, the variety must be sufficiently uniform in its relevant characteristics (Article 8);

- Stability – the variety is stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle (Article 9).

Where plant varieties resulting from synthetic biology techniques fulfil these criteria, the breeder has the possibility to obtain a breeder's right, which includes that the following require the authorization of the breeder (i) production or reproduction (multiplication); (ii) conditioning for the purpose of propagation; (iii) offering for sale; (iv) selling or other marketing; (v) exporting; (vi) importing, and (vii) stocking for any of these purposes (Article 14 UPOV Convention). The breeder's right is granted by an individual UPOV member.

In addition, the breeder's right can be obtained for varieties which are essentially derived from the protected variety, a variety that requires the repeated use of the protected variety, or a variety which was not clearly distinguishable from the protected variety (Article 14, paragraph 5(a)). This may be relevant for synthetic biology, as the UPOV Convention states that essentially derived varieties may be obtained for example by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing, or transformation by genetic engineering (Article 14, paragraph 5(c)).

To qualify for the breeder's right, essentially derived varieties need to (i) be predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety; (ii) be clearly distinguishable from the initial variety; and (iii) except for the differences which result from the act of derivation, conform to the initial variety in essential characteristics that result from the genotype or combination of genotypes of the initial variety. Where both the essentially derived variety and the initial variety are protected by breeders' rights, the activities listed in Article 14, paragraph 1 with regard to the essentially derived variety require the authorization of both breeders (UPOV, 2009a).

Exceptions to the breeder's right

Article 15 to the UPOV Convention provides for certain exceptions to the breeder's right. According to paragraph 1, compulsory exemptions address: (i) acts which are both private and for non-commercial purposes; (ii) the use of a protected variety for experimental purposes; and (iii) the use of protected varieties for the purpose of breeding new plant varieties. The commercialization of a new variety would not require the authorization of the breeder of the protected variety, except where the new variety is an essentially derived variety, a variety that requires the repeated use of the protected variety or was a variety which was not clearly distinguishable from the protected variety in accordance with Article 14, paragraph 5 of the UPOV Convention. UPOV members may, under an optional exception in Article 15, paragraph 2 of the UPOV Convention, allow farmers to save harvested material for further propagation under certain circumstances (UPOV, 2009b). While the TRIPS Agreement leaves open the option of excluding from the scope of patentability inventions whose commercial exploitation needs to be prohibited to address these concerns, Article 17 of the UPOV Convention allows its members to

restrict the free exercise of a breeder's right for reasons of public interest.

9.4.2. Commerce and trade

(a) The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)

The Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization (SPS Agreement) is part of the system of multilateral trade rules of the World Trade Organization (WTO). The SPS Agreement attempts to strike a balance between, on one hand, reaffirming the rights of WTO members to adopt and enforce measures that are necessary to protect human, animal or plant life or health, and, on the other hand, making sure that these measures are not excessively trade restrictive. The SPS Agreement applies to all sanitary and phytosanitary measures that directly or indirectly affect international trade (Article 1 SPS Agreement).

Sanitary or phytosanitary measures

Sanitary or phytosanitary measures can take many forms, including laws, decrees, regulations, requirements; testing, inspection, certification and approval procedures; quarantine treatments; requirements associated with the transport of animals or plants; sampling procedures; and methods of risk assessment. The SPS Agreement defines sanitary and phytosanitary measures as any measure applied with one of the following objectives (Article 1, paragraph 2 in conjunction with Annex A, paragraph 1 SPS Agreement):

- To protect animal or plant life or health within the territory of the member from risks arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms or disease-causing organisms;

- To protect human or animal life or health within the territory of the member from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs;

- To protect human life or health within the territory of the member from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; or

- To prevent or limit other damage within the territory of the member from the entry, establishment or spread of pests.

WTO members have the right to take sanitary and phytosanitary measures that are necessary for the protection of human, animal or plant life or health, even if these measures result in trade restrictions. However, these measures have to be consistent with the provisions of the SPS Agreement (Article 2, paragraph 1 SPS Agreement). Requirements include, for example, that the measures must be based on scientific principles, must not unjustifiably discriminate in their effect on other WTO members' exports, and must not be more trade-restrictive than is necessary to achieve the appropriate level of sanitary or phytosanitary protection (Articles 2, 3 and 5; SPS Agreement).

The SPS Agreement encourages WTO members to harmonize their sanitary and phytosanitary measures on the basis of international standards, guidelines and recommendations, since harmonization reduces costs for producers and traders and generally facilitates trade. Sanitary and phytosanitary measures that conform to international standards, guidelines or recommendations are deemed to be necessary to protect health and are presumed to be consistent with the SPS Agreement. For such measures that conform to international standards, WTO members thus e.g. do not have to provide a scientific justification.

The SPS Agreement explicitly recognizes the international standards, guidelines and recommendations developed by three organizations: for food safety, the Codex Alimentarius Commission; for animal health and zoonoses, the relevant international standards, guidelines and recommendations developed by the World Organisation for Animal Health (OIE); for plant health, those developed by the International

Plant Protection Convention (IPPC). For matters not covered by these three organizations, there is a possibility that the Committee on Sanitary and Phytosanitary Measures under the SPS Agreement could identify standards developed by other relevant international organizations, but so far there has never been a proposal to recognize another standard-setting body.

If no relevant international standard exists, or when a WTO member wishes to deviate from an existing international standard, measures have to be based on a risk assessment. In this context, a risk assessment is defined as the evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing member according to the sanitary or phytosanitary measures which might be applied, and of the associated potential biological and economic circumstances. Risk assessments must take into account risk assessment techniques developed by the relevant international organizations. Risk assessments also have to take into account available scientific evidence; relevant processes and production methods; prevalence of specific diseases or pests; existence of pest- or disease-free areas; relevant ecological and environmental conditions; and quarantine or other treatment. In assessing the risk to animal or plant life or health and determining the measure to be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk, WTO members are also required to take into account certain economic factors, namely: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing member; and the relative cost-effectiveness of alternative approaches to limiting risks (Article 5(3)).

In situations where relevant scientific evidence is insufficient to carry out a risk assessment, the SPS Agreement allows members to adopt provisional sanitary and phytosanitary measures on the basis of the available pertinent information, including that from relevant international organizations and from measures applied by other members. When they adopt such provisional measures, members have to try to obtain additional information to allow them to carry out a risk assessment and review the provisional measure within a reasonable period of time.

Pests, diseases, disease-carrying organisms or disease-causing organisms

Sanitary and phytosanitary measures may be relevant to components, organisms and products resulting from synthetic biology if they result in pests, diseases, disease-carrying organisms or disease-causing organisms with negative impacts on human, animal or plant life or health. The SPS Agreement, however, does not define "diseases, disease-carrying organisms or disease-causing organisms", nor "pests". A footnote clarifies that, for the purpose of the definitions of the SPS Agreement (Article 1, paragraph 2 in conjunction with Annex A SPS Agreement), "pests" include weeds.

The intentional or unintentional release of components, organisms or products resulting from synthetic biology may lead to biosafety or biosecurity concerns. Depending on the circumstances, they could be considered to pose risks to animal or plant life or health, through ecosystem-level impacts or the transfer of synthetic DNA. WTO members may take sanitary and phytosanitary measures to address these risks in accordance with the objectives and requirements summarized above under "Sanitary or phytosanitary measures".

A WTO dispute between the USA and the European Communities (EC) concerning "Measures affecting the Approval and Marketing of Biotech Products" (EC-Biotech case) concerned measures by the EC which the USA claimed were contrary to its obligations under a number of international agreements, including the SPS Agreement. A detailed review of the dispute can be found in the original Technical Series document (No. 82) on synthetic biology published in 2015. The dispute highlights that organisms resulting from synthetic biology could, depending on the specific case, be considered as causing risks to animal or plant life or health arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms or disease-causing organisms. It may be noted, however,

that the measures were considered to be contrary to the SPS Agreement on other grounds.[162]

Additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs

Components, organisms and products resulting from synthetic biology could arguably also be addressed through measures to protect human or animal life or health within the territory of a WTO member from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs (Annex A, paragraph 1(b)).

The WTO Panel on the EC-Biotech dispute also provided guidance for the case of genetically modified organisms. It held that "*a genetically modified crop grown for the explicit purpose of providing food to animals*", and in particular to farmed animals, would qualify as a "feedstuff". A genetically modified crop that has been grown for a different purpose, but is eaten by animals, including wild fauna, can be considered to be a "food" for that animal. This would include, for example, pollen of the genetically modified crop which is consumed by insects, and genetically modified plants consumed by non-target insects, deer, rabbits or other wild fauna. The panel stated that "*genetically modified seeds used for sowing purposes could also be considered animal 'food', for instance if these seeds are spilled next to a field or on a farm and are subsequently eaten by birds, etc.*".

With regard to the definition of "additives", the Panel held that "genes, intentionally added for a technological purpose to genetically modified plants that are eaten or being used as an input into processed foods, can be considered "additives in foods" within the meaning of Annex A(1)(b). This should not be construed to mean, however, that all genes of a plant that is eaten or being used as input into processed foods could be classified as "additives" (World Trade Organization, 2006).

The Panel stated further that "contaminants" must be interpreted so as to have a meaning that differs from the meaning of the term "additive" and that the decisive element in this regard is that the presence of the substance which is said to "infect or pollute" is unintentional. Genes intentionally added to genetically modified plants that are eaten or used as inputs into processed foods would not be "contaminants" in and of themselves. Also, substances such as proteins which are produced by genetically modified plants, and which are intended, should not be considered to be "contaminants". However, proteins produced through the unintended expression of modified genes in agricultural crops may be considered "contaminants" within the meaning of Annex A(1)(b) if these proteins "infect or pollute" (World Trade Organization, 2006).

With regard to the definition of "toxin" the Panel stated that "*a poisonous substance which is produced during the metabolism or growth of a genetically modified crop could qualify as a "toxin" within the meaning of Annex A(1)(b)*". It noted that "*for an SPS measure to be covered by Annex A(1)(b), the toxin which gives rise to risks for human or animal life or health would have to be present in "foods, beverages or feedstuffs"*, but recalled at the same time that "*a genetically modified plant which is grown in a field may be eaten as food by wild fauna*". The Panel also stated that food allergens at issue in the dispute can be considered as "toxins". The Panel did not give any guidance as to the interpretation of the term "disease-causing organisms" (World Trade Organization, 2006).

Case-by-case assessments would be necessary to determine whether any components, organisms or products of synthetic biology would be covered by Annex A(1)(b). At this point, applications of synthetic biology do not seem to be focusing on developing food crops for human use, but the potential for synthetic biology to enhance agricultural efficiency and lessen its environmental impacts is often invoked. Where organisms resulting from synthetic biology could be accessed by wild fauna, they may qualify as "feedstuffs". For example, outdoor ponds of algae resulting from synthetic biology techniques may be accessible to wildlife (Snow & Smith, 2012). Whether any components, organisms

162 https://www.wto.org/english/tratop_e/dispu_e/cases_e/1pagesum_e/ds291sum_e.pdf.

or products of synthetic biology that qualified as a food, beverage, or feedstuff would also be considered an additive, contaminant or toxin would, again, require a case-by-case assessment, taking into account the intended expressions of synthetic genetic sequences.

(b) The International Plant Protection Convention (IPPC)

The International Plant Protection Convention (IPPC) promotes action to protect plants and plant products from the spread of pests and sets out measures to control plant pests (see Article I IPPC).[163] The latest version of the convention entered into force in 2005; it has 184 Parties.

Overview of main provisions

The main provisions of the IPPC include the requirement for each Party to establish a national plant protection organization with a specified mandate (Article IV IPPC) and to make arrangements for the issuance of phytosanitary certificates (Article V IPPC). Further, Parties may require, under certain conditions, phytosanitary measures for quarantine pests and regulated non-quarantine pests (Article VI IPPC). Parties also have sovereign authority to regulate, in accordance with applicable international agreements, the entry of plants and plant products and other regulated articles with the aim of preventing the introduction and/or spread of regulated pests into their territories (Article VII, paragraph 1 IPPC). To this end, Parties may:

- Prescribe and adopt phytosanitary measures concerning the importation of plants, plant products and other regulated articles, including, for example, inspection, prohibition on importation, and treatment;

- Refuse entry or detain, or require treatment, destruction or removal from the territory of the contracting party, of plants, plant products and other regulated articles or consignments thereof that do not comply with the phytosanitary measures prescribed or adopted under subparagraph (a);

- Prohibit or restrict the movement of regulated pests into their territories;

- Prohibit or restrict the movement of biological control agents and other organisms of phytosanitary concern claimed to be beneficial into their territories.

In order to minimize interference with international trade, Parties have to undertake these activities in conformity with a set of requirements provided in Article VII, paragraph 2.

In Article X, Parties agree to cooperate in the development of international standards which they should take into account when undertaking activities related to the convention. In accordance with these provisions, the international framework for plant protection includes International Standards for Phytosanitary Measures (ISPMs). The adopted standards under the IPPC[164] provide guidance to its Parties on phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade, with specific standards covering not only pest risk analysis but also import and export systems, post-border controls and surveillance and reporting on pests and diseases.

Phytosanitary measures

The International Plant Protection Convention defines phytosanitary measures in Article II as any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of pests. Pests, in turn, are defined as any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products. Plants are defined as living plants and parts thereof, including seeds and germplasm. Plant products are defined as unmanufactured material of plant origin (including grain) and those manufactured products that, by their nature or that of their processing, may create a risk for the introduction and spread of pests.

While the primary focus of the International Plant Protection Convention is on plants and plant

163 Secretariat of the International Plant Protection Convention (1999).
164 Available at www.ippc.int/core-activities/standards-setting/ispms#block-agenda-items-list.

products moving in international trade, it also covers research materials, biological control organisms, germplasm banks, containment facilities and anything that can act as vectors for the spread of plant pests (e.g. containers, packaging materials, soil, vehicles, vessels and machinery). Regulated articles comprise any plant, plant product, storage place, packaging, conveyance, container, soil and any other organism, object or material capable of harbouring or spreading pests, deemed to require phytosanitary measures, particularly where international transportation is involved (see also Article I, paragraph 3 IPPC).

ISPM No. 11, "Pest risk analysis for quarantine pests", addresses LMOs. It notes the types of LMOs that a national plant protection organization (NPPO) may be asked to assess for phytosanitary risk:

- Plants for use (a) as agricultural crops, for food and feed, ornamental plants or managed forests; (b) in bioremediation (as an organism that cleans up pollution); (c) for industrial purposes (e.g. production of enzymes or bioplastics); (d) as therapeutic agents (e.g. pharmaceutical production);
- Biological control agents modified to improve their performance in that role;
- Pests modified to alter their pathogenic characteristic and thereby make them useful for biological control (see ISPM No. 3 (Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms));
- Organisms genetically modified to improve their characteristics such as for biofertilizer or other influences on soil, bioremediation or industrial uses.

Annex 3 to ISPM No. 11 provides guidance for "Determining the potential for a living modified organism to be a pest". It clarifies that for phytosanitary risks related to gene flow, the LMO is acting more as a potential vector or pathway for introduction of a genetic construct of phytosanitary concern, than as a pest in and of itself. Therefore, the term "pest" should be understood to include the potential of a LMO to act as a vector or pathway for introduction of a gene presenting a potential phytosanitary risk. Annex 3 to ISPM No. 11 contains a list of potential phytosanitary risks from LMOs. All these risks may apply, to varying degrees, to components, organisms and products resulting from synthetic biology (FAO, 2019a).

Other ISPMs which have been identified as relevant to LMOs (SCBD, 2012), and which therefore may in some cases be relevant to components, organisms and products resulting from synthetic biology, include:

- ISPM No. 12: Phytosanitary certificates (revised 2017);
- ISPM No. 7: Phytosanitary certification systems (revised 2016);
- ISPM No. 3: Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms (revised 2017);
- ISPM No. 20: Guidelines for a phytosanitary import regulatory system (revised 2019);
- ISPM No. 23: Guidelines for inspection (revised 2019).

(c) World Organisation for Animal Health

The World Organisation for Animal Health was founded in 1924 as the Office International des Epizooties (OIE) to provide international cooperation and coordination against the spread of animal diseases. Ninety years later, the core mandate of the organization has been expanded to become the improvement of animal health worldwide.

The OIE standards, recognized by the SPS Agreement as the international standards for animal health and zoonoses, are published as the OIE Animal Health Codes (Terrestrial Animal Health Code and Aquatic Animal Health Code) and the OIE Manuals (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals and Manual of Diagnostic Tests for Aquatic Animals). These international

standards cover a wide range of animal health and veterinary public health matters. They include the obligation to issue notifications, undertake import risk analyses, surveillance, disease prevention and control measures, establish trade requirements for animals and animal products, and require the use of diagnostic tests and vaccines and others.

Sanitary measures

A sanitary measure under the OIE means a measure, such as those described in various chapters of the Terrestrial Code, destined to protect animal or human health or life within the territory of the member country from risks arising from the entry, establishment and/or spread of a hazard. A hazard is defined in the Terrestrial Code as a biological, chemical or physical agent in, or a condition of, an animal or animal product with the potential to cause an adverse health effect.

As this definition is quite broad, components, organisms and products resulting from synthetic biology techniques could potentially fall thereunder. As mentioned previously, although current applications of synthetic biology are mostly in microorganisms, synthetic biology research in mammalian and other eukaryotic cells is making rapid progress. OIE standards may be relevant to synthetic biology techniques both in terms of synthetic biology helping to develop vaccines and therapies for animal diseases and in terms of possibly producing adverse health effects.

(d) Codex Alimentarius

The Codex Alimentarius Commission is a joint initiative of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) that was set up to establish international standards on foods.[165]

The Codex Alimentarius is a collection of internationally adopted food standards presented in a uniform manner. These are developed in order to attempt to ensure that products meet internationally accepted minimum quality levels, are safe, and do not present a health hazard. Standards are prescribed for individual foods and food groups, and general standards have also been adopted. In addition to specific standards, the Codex also includes "related texts". Related texts include advisory instruments: statements of principle, codes of practice, guidelines and codes of technological practice. Some of these instruments apply to food and food products that have been derived from synthetic biology techniques.

Standards adopted by the Codex Alimentarius Commission are not legally binding on Codex member States. Countries and organizations that are members of the World Trade Organization (WTO), however, have a general obligation under the SPS Agreement to base their sanitary or phytosanitary measures on international standards, guidelines or recommendations, where they exist, for the purpose of harmonizing these measures on as wide a basis as possible (Article 3, paragraph 1 SPS Agreement). Annex A to the SPS Agreement defines the term "international standards, guidelines and recommendations" to mean, in the context of food safety, the standards, guidelines and recommendations established by the Codex Alimentarius Commission (paragraph 3(a)). These standards are generally the basis of food safety regulation, which includes foods derived from LMOs.

Documents relevant to components, organisms and products resulting from synthetic biology include, for example:[166]

- Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (2011);
- Compilation of Codex texts relevant to the labelling of foods derived from modern biotechnology (2011);
- Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (2008) and its annex on Food Safety

165 For an introduction to the Codex Alimentarius see http://www.codexalimentarius.org/about-codex/en/.
166 These documents are available online at www.codexalimentarius.org/standards/list-of-standards/.

Assessment of Foods Derived from Recombinant-DNA Plants Modified for Nutritional or Health Benefits;

- Guideline for the Conduct of Food Safety Assessment of Foods Produced using Recombinant-DNA Microorganisms (2003);

- Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals (2008).

These standards may be relevant if components, organisms and products resulting from synthetic biology are used as foods. The term "modern biotechnology" has the same definition under the Codex Alimentarius and the Cartagena Protocol on Biosafety. For an analysis, see therefore subsections 8.1.4 and 8.2.1 above.

E. Observations, Analyses and Conclusions

In this section, we draw on the international landscape mapping presented in previous sections (8 and 9) in order to highlight apparent gaps and overlaps associated with the governance of synthetic biology, as well as potential challenges and opportunities for synergies and cooperation.

It is worth noting that when doing an analysis of potential gaps and overlaps associated with the governance of synthetic biology, it is recognized that the wide range of applications of synthetic biology, as well as its cross-cutting nature, makes it difficult to draw a clear line of action or intervention for some international initiatives. Overlaps can be expected and are not necessarily duplication. Instead they could be related to the fact that a particular aspect of synthetic biology could be within the scope of more than one initiative and considered through different lenses and scope.

This section also contains the overall conclusions of the document.

10. Challenges, gaps and/or overlaps associated with synthetic biology governance

Synthetic biology is often referred to as a multidisciplinary area of research and its underlying complexities create challenges for its effective governance. The lack of consensus on an agreed definition of synthetic biology, the unclear distinction between synthetic biology and genetic engineering, the numerous and ever-evolving areas of synthetic biology research, and the wide array of their applications and potential impacts illustrates this complexity. These diverging views on which techniques or applications could be considered to be synthetic biology poses challenges at various levels, ranging from governance to regulation. However, there is interest in capitalizing on the new technologies and applications of synthetic biology to maximize the potential benefits while minimizing risks.

The diversity of techniques and applications also creates challenges in assessing potential gaps and overlaps associated with the work done under the Convention and its Protocols and under other international treaties, laws, processes and initiatives which could have potential implications for the international governance of synthetic biology. As far as today's applications of synthetic biology are concerned, the authors did not identify any topic that would not be covered by one or more of the governance regimes or processes of rule-making described in sections 8 and 9. No apparent gaps were highlighted, although some may argue that the absence of a specific governance or process of rule-making on an international scale to "regulate" synthetic biology represents a major gap in itself. Of course, this may change as techniques and applications continue to evolve and therefore requires careful monitoring.

Rather, an extensive collection of regulatory instruments and mechanisms apply to all or some forms of synthetic biology depending on the application. Diverging mandates create particular challenges in balancing risks and potential benefits arising from specific synthetic biology applications; for example, in the context of engineered gene drives, how are biosafety considerations under the Convention and its Protocols to be combined with or balanced with the implications for human health as considered by WHO? It is also important to acknowledge that the rapid pace of technological development can present a challenge to definitions and also the potential scope of the Convention and its Protocols. In a similar manner, it is also important to consider that most regulatory mechanisms discussed in the present document were developed before some of the tools that enable synthetic biology and even the term itself became widely used. Therefore, they may lack the necessary scope and scale to address some

of the potential impacts that some applications of synthetic biology may present.

However, within this multifaceted regulatory landscape, the broad scope of the Convention and its Protocols, focusing on conservation, sustainable use and fair and equitable benefit-sharing associated with biodiversity, has far-reaching implications for the international governance of synthetic biology. Recognition of the Convention as "*the primary international forum deliberating the regulation of synthetic biology*" (Keiper & Atanassova, 2020) reflects over a decade of substantive decision-making by the Conference of the Parties explicitly addressing synthetic biology on issues such as its relevance to the objectives of the Convention and its Protocols, biosafety risks associated with LMOs, implications concerning access and benefit-sharing, and the participation of indigenous peoples and local communities, among others.

In parallel, other instruments and organizations, such as WHO, CITES and IUCN, have in recent years also developed their own substantive programmes of work related to the governance of synthetic biology, and appear to be significantly informed by the extensive and cross-cutting work undertaken to date under the Convention and its Protocols. In a similar manner, the discussions under the Convention and its Protocols are also influenced by the global discussions and deliberations under these instruments and organizations, in particular where there are issues of mutual interest or opportunities for synergies. It is, however, important to note that the discussions under these and other instruments and initiatives are contextualized and relate to specific aspects of synthetic biology rather than the overarching elements. Nonetheless, as a growing number of synthetic biology applications advance from upstream research to market-ready deployments, issues of scope and potential impact of such applications can be considered in context, on a case-by-case basis. Such context is necessary to evaluate whether the synthetic biology applications result in an organism, product or component, to consider risk assessment and mitigation approaches in a tailored manner, and to identify relevant governance implications under international treaties, laws, processes and initiatives.

Finally, there is a need for a more holistic approach for international governance of synthetic biology and the opportunity for this to leverage the existing initiatives and coordination mechanisms available under the Convention and its Protocols.

10.1. The state of knowledge associated with tools, technologies, and applications of synthetic biology

The cost of sequencing and synthesis of nucleic acids has decreased drastically and there is access to more genetic information and more powerful genetic engineering capabilities than ever before. However, despite the development of some high-value products, and the recognition that synthetic biology is at the cusp of many major breakthroughs, some authors argue that there is still a perception that synthetic biology is not yet delivering on its promise (El Karoui et al., 2019).

There is incomplete understanding of how nature works. This makes it difficult to apply the Design-Build-Test-Learn cycle to the generation of synthetic biological products, whatever the production platform (microbial, plants, or mammalian cells), if the platform itself is not well understood. This incomplete understanding is also related to challenges faced in the risk assessment of some synthetic biology applications.

Similarly, many synthetic biology technologies strongly rely on computing and informatics tools that help the design process. Instruments able to measure and characterize outputs, assisted by progress in robotics and automation, and the application of machine learning approaches to analyse the data generated are increasingly needed. Advancing the field requires novel approaches for modelling bioprocesses that follow different biochemical and biophysical rules that still allow simulation with the computational power available today. The predictive models that are needed require a critical level of prior knowledge that typically researchers do not have, e.g. about the biological components and their

interactions. The ability to pool data and models, essential for improving accuracy and reproducibility, is challenging without interoperability around biosystem modelling and some degree of "standardization" especially around DNA design.

10.2. Risk of harm

(a) The risk of harm to the environment, biodiversity and human welfare

As the primary international instrument governing the conservation and sustainable use of biological diversity, the Convention on Biological Diversity establishes a framework for handling biotechnology. The Cartagena Protocol on Biosafety elaborates a framework for assessing the potential risk of harm to the environment and biodiversity, and as appropriate, to human health, that may be caused by the transfer, handling and use of LMOs, which may also apply to transboundary movement of LMOs that are derived from synthetic biology. Additionally, the Nagoya Kuala – Lumpur Supplementary Protocol provides international rules and procedures in the field of liability and redress relating to LMOs, and the Conference of the Parties has reaffirmed the importance of participatory decision-making by indigenous peoples and local communities and their adequate consultation in the context of biosafety. The provisions on biosafety and the requirement to take into account the precautionary approach in decision-making are particularly relevant to the governance of synthetic biology.

The framework established under the Convention, which includes biosafety considerations, is complemented by other international instruments focusing on biosecurity and containment which also have implications for harm to the environment and biodiversity. These include the Biological Weapons Convention, which would apply to the use of components, organisms and products resulting from synthetic biology techniques for hostile purposes or in armed conflict, as well as the Environmental Modification Convention, which would apply if synthetic biology techniques were developed to modify weather for the purposes of inducing damage or destruction. So far, these appear to have devoted limited attention to evaluating the risk of harm arising from synthetic biology techniques. However, given the dual-use concerns associated with synthetic biology and biotechnology more generally, a closer examination concerning biosecurity risks and the mitigation of harm to the environment, biological diversity and human welfare appears likely and this will likely take into consideration the substantial body of work to date under the Convention and its Protocols, with the potential for greater collaboration in the future.

General principles of international law such as the duty to avoid transboundary harm and the need to conduct an environmental impact assessment, together with the rules of State responsibility, can also provide some guidance relevant to addressing potential negative impacts resulting from the application of synthetic biology techniques. However, it can be argued that these would still form an incomplete basis to address all potential positive and potential negative impacts given the range of uncertainties inherent to emerging applications, especially in the absence of specific guidance. Additionally, although self-regulation by the synthetic biology community is no substitute for appropriate international regulation and governance of synthetic biology, it may also play an important role in mitigating the risk of harm, whether to the environment and biodiversity or to human welfare more generally. So-called "dual-use" technologies in particular have the potential to undermine public confidence regarding the safe use of synthetic biology techniques. Uncertainty and heightened sensitivity associated with the possibility that synthetic biology techniques could be deliberately misused or misappropriated could hamper the development of useful applications. Scientists, their host institutions and funding bodies should consider whether their planned research could result in harm or be misused. Measures that reduce the likelihood of misuse and its consequences should be implemented and clearly communicated.

(b) The risk of harm related to human health

The Convention on Biological Diversity and its biosafety-related Protocols enable Parties to take into account risks to human health as part of the

evaluation and mitigation of risks associated with the use and release of LMOs.

There are other international organizations (e.g. WHO) with mandates that are directly related to human health issues. This suggests that it would be beneficial for the international organizations with overlapping and/or complementary mandates to collaborate in relation to applications of synthetic biology which have implications for human health. In a containment context, for instance, the WHO's Laboratory Biosafety Manual provides guidance regarding biosafety in the context of clinical and public health. Other WHO indications also have implications for biosecurity, such as guidance concerning the handling of smallpox to mitigate the risk of its re-emergence or inappropriate use. Given the possibility that eradicated diseases could potentially be manufactured using synthetic biology techniques, such safeguards could be seen as complementary to the Cartagena Protocol. Finally, in a public health context concerning for instance the environmental releases of synthetic biology health-oriented applications (e.g. LM mosquitoes containing engineered gene drives), potential interactions could arise as different organizations (e.g. Convention and WHO) assess the application through different lenses and in accordance with their respective mandates and objectives.

The potential interplay associated with the risk of harm related to human health under the Convention and its Protocols could also arise in relation to international instruments and initiatives governing phytosanitary measures, including quarantine and biosecurity measures applied to protect human, animal or plant life or health from risks arising from the introduction, establishment and spread of pests and diseases as well as from risks arising from additives, toxins and contaminants in food and feed.

10.3. Conservation

Some considerations under the Convention and its Protocols have focused on their biosafety provisions governing risk assessment and mitigation of adverse effects on the conservation and sustainable use of biological diversity. However, the Convention's mandate related to conservation is of course much broader than biosafety, and there is also potential for interactions with international instruments and initiatives which also focus on the conservation of biodiversity as a key objective. Such interactions are readily apparent with IUCN, for instance, given its primary focus on nature conservation and sustainable development. Unlike the Convention and its Protocols, IUCN is not established by contracting Parties under an international treaty; however, given the strong participation of the conservation community, and the IUCN work on synthetic biology initiated in 2016, including its technical assessment on synthetic biology undertaken in 2019 in relation to conservation, there are opportunities for synergies with the activities under the Convention and its Protocols focusing on synthetic biology governance. Actions from any of these bodies are likely to influence and have an impact on those of the others.

10.4. Access and benefit-sharing

Synthetic biology also raises a number of questions with regard to access and benefit-sharing under the Convention and its Nagoya Protocol. This includes whether digital sequence information accessed for use in synthetic biology can be considered "genetic resources" or "genetic material". The process that is currently under way under these treaties to resolve a divergence of views among Parties regarding the benefit-sharing from the use of digital sequence information on genetic resources may have implications for synthetic biology organisms and applications developed through the use of digital sequence information. Similar challenges concerning access and benefit-sharing in the context of digital sequence information (however called) and synthetic biology are being evaluated in the context of other international instruments and initiatives governing access to genetic resources. These include, among others, the International Treaty on Plant Genetic Resources for Food and Agriculture, the Pandemic Influenza Preparedness (PIP) Framework of WHO, and an international agreement, under development, on the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction under UNCLOS, all of which are likely to consider the outcomes of the process

under the Convention and its Nagoya Protocol to inform their own deliberations.

10.5. Commerce and trade

To the extent that the Convention and its Protocols govern the environmental release and transboundary movement of synthetic biology organisms, as well as the sharing of benefits that may be associated with the utilization of genetic resources in their development, this can be seen to have implications for commerce and trade. Several other international agreements regimes and initiatives exist which, in general, may have implications associated with commerce and trade. CITES is particularly relevant in the context of synthetic biology as it is evaluating "bioengineered specimens", "specimens produced through biotechnology" or "wildlife products produced from synthetic or cultured DNA", as part of its evaluation of whether trade in synthetic specimens of CITES-listed species that closely mimic both the physical appearance and biological characteristics of their wildlife counterparts should be regulated.

The TRIPS Agreement and the UPOV Convention, which govern intellectual property protection, do not have any known programme of work addressing synthetic biology specifically. However, given the extent to which they underpin intellectual property considerations in international trade and commerce, they are likely to have significant implications for the governance of synthetic biology. Similarly, international instruments and initiatives governing phytosanitary measures, including quarantine and biosecurity measures, have implications for commerce and trade related to synthetic biology organisms and products. Accordingly, the international framework related to phytosanitary measures established by the SPS Agreement and IPPC, the OIE standards, and related instruments such as those developed by the Codex Alimentarius, which establishes standards on food, are likely to have implications for the governance of synthetic biology.

An additional challenge specifically relates to the capability to detect and identify synthetic biology applications. Many potential synthetic biology organisms may not be easily or feasibly detectable (e.g. if the changes to the genome cannot be differentiated from mutations that may occur naturally) or identifiable in the final/commercial product. This can create a regulatory and a technical challenge for the overall system for managing synthetic biology, especially concerning transboundary movements (see subsection 6.2.5).

10.6. Governance approaches and public acceptance

As relatively few synthetic biology applications have obtained authorization for wide release, relatively few data from their biotic interactions and any presence in ensuing supply chains have been collected, including those that may reflect benefits. Thus, the range of potential impacts on the conservation and sustainable use of biodiversity as a whole remains largely hypothetical/speculative and case-specific, informed by limited small-scale experiments, mostly in the laboratory, and by previous experience with LMOs with similar traits. Further, the evaluation and/or quantification of any benefits has not been visible in decision-making under many, but not all, regulatory systems; a situation exacerbated by the lack of agreed international standards with respect to the types of data to collect, and how, for each type of application. As a consequence, any potential benefits of each application should, by necessity, be considered on a case-by-case basis and not be extrapolated to all uses of each application, as the socioeconomic and cultural considerations will be context-specific. A theme emerging from the literature which considers challenges in public acceptance and concerns associated with applications of synthetic biology (see section 5) is that the evaluation of social, economic, and cultural considerations, as well as inclusive decision-making and community engagement, may be as important as the consideration of potential impacts on biodiversity conservation and sustainable use during decision-making and governance of synthetic biology applications. Inclusive decision-making and community engagement provide an opportunity to evaluate and address concerns, strengthen governance and steer responsible research and use of synthetic biology applications that pose potential risk of harm to biodiversity.

One approach that has already been championed concerns FPIC, which has been prominent in decisions made under the Convention (see subsections 5.1.2 and 8.1.7 above). Parties must therefore ensure that when decisions concerning synthetic biology have implications for IPLCs, particularly related to environmental release, the issue of FPIC including its practical implementation is given critical importance and inclusive and participatory approaches to decision-making are adopted. Other international instruments and initiatives focusing on IPLCs and participatory decision-making through their FPIC also complement the framework established under the Convention. Alongside the Convention, the Indigenous and Tribal Peoples Convention and the United Nations Declaration on the Rights of Indigenous Peoples safeguard the rights and interests of IPLCs and contribute to evolving principles underpinning FPIC and its implementation. Recent decisions by the Conference of the Parties to the Convention on Biological Diversity have explicitly recognized the importance of FPIC of IPLCs in the context of the environmental release of LMOs containing engineered gene drives; however, challenges remain in the translation of FPIC principles into effective and standardized protocols for community consultations and participatory decision-making.

In addition to community engagement (indigenous or otherwise), cooperation between biosecurity experts, social scientists and practitioners to ensure a "building of bridges" early in the technology development and forecasting stages is also considered an essential component for building public acceptance of synthetic biology applications (Trump et al., 2020). A review of a broad collection of synthetic biology literature from 2000-2016 focuses on the co-development of physical and social science communities throughout synthetic biology's earliest stages of development and observes how this has helped overcome significant challenges of the technology's risk assessment, governance, and public engagement needs, concluding that an interdisciplinary approach is necessary to foster sustainable, risk-informed, and societally beneficial technological advances moving forward (Trump et al., 2019).

Another approach gaining traction is "Responsible Research and Innovation" which calls on researchers to assess societal implications of emerging technologies to optimize the alignment of expected outcomes with the needs and values of society more broadly than human health and the environment. This includes, for example, evaluating and reducing downstream harms that might expose developers, companies and governments to expensive clean-up and/or insurance efforts (Trump et al., 2020). Its adoption has also been recommended specifically in the context of technology assessment for synthetic biology to ensure that it not only takes into consideration technical aspects, but also includes societal and ethical issues (Gregorowius & Deplazes-Zemp, 2016; Macnaghten et al., 2016). Comparable approaches are illustrated by recommendations for the use of so-called "Governance Coordinating Committees" and principles of transparency, accountability, integrity and capacity in the governance of, for instance, the release of engineered gene drive mosquitoes, as one strategy to address the lack of precedent and to address regulatory gaps and overlaps (Kelsey et al., 2020).

There is evidence of scientific research addressing community engagement in field trials, for example concerning LMOs containing an engineered gene drive for malaria control (Resnik, 2018). Other efforts are proving that technology development could be shaped at an early stage by engagement with community members. This is the case of the development of gene-edited mice with heritable resistance to tick-borne diseases, where the researchers have considered the views, preferences and needs of the community in designing their approach. This engagement process, engaging community members into sharing suggestions and concerns, has guided the researchers in making project decisions (Buchthal et al., 2019). Another often-cited example of community engagement concerns the release of Wolbachia-infected (non-LM) mosquitoes in northern Australia to eliminate dengue, as a model for the release of LM mosquitoes containing engineered gene drives (Kolopack et al., 2015).

In order to enable or improve community engagement in the development and deployment of synthetic biology research, one of the primary challenges involves developing relationships with communities to the extent that their involvement is encouraged and maintained. This is especially key when working with IPLCs. Assisting the process would be the creation of a community advisory board to be involved in the development of the research project and application in a manner that helps overcome any differences between the community and the involved researchers and developers, and which could help in guiding research towards approaches that are acceptable by the communities most likely to be impacted. In the absence of widely agreed-upon governance guidelines or support for more optimal deliberative processes, the developers of a technology seeking consent to release a synthetic biology organism may also serve as a community's source of expertise and information. Such an "advice and consent" relationship raises the possibility of a real or apparent conflict of interest. Ideally in these cases, governance plans should incorporate expertise and perspectives that are independent or at least free of conflicts of interest, as well as transparent, inclusive, and based on balanced deliberations. This situation is further exacerbated by the complex technical and scientific language used in the context of synthetic biology. For resource constrained, low science literate communities, this is a real concern. Communication and outreach material need to be translated and conceptualized for meaningful public participation and FPIC. An early example of such material is the common glossary co-developed by collaboration between local communities, linguists and researchers focusing on translations into local languages of key terms of malaria vector control, ranging from genetics (gene, chromosome, DNA), to entomology (mosquito, larvae, collection, swarming), laboratory (containment, insectary, biosecurity), through more common engagement language (consent, engagement, community acceptance) (Chemonges Wanyama et al., 2021).

Socially informed scientific initiatives need broader support from the scientific community, funders and policymakers. Examples include the Scientific Citizenship Initiative[167] at Harvard University in the USA, which trains scientists to align their research with societal needs. The Summer Internship for Indigenous Peoples in Genomics offers genomics training that focuses on integrating indigenous cultural perspectives into gene studies. The AI Now Institute[168] at New York University (USA) has initiated a holistic approach to artificial-intelligence research that incorporates inclusion, bias and justice. Editing Nature[169] provides platforms that integrate scientific knowledge with diverse cultural world views to foster the responsible development of environmental genetic technologies. Also, Sheila Jasanoff, founding director of the Program on Science, Technology, and Society at Harvard Kennedy School, who has led calls for a "global observatory" to promote exchange across disciplinary and cultural divides on gene editing through an international network of scholars and organizations, has also suggested the approach should be used to address emerging technologies more broadly.[170]

As we think about synthetic biology in the future, the challenge is to create a framework that fosters scientific creativity and allows research and product development to move ahead, while acting responsibly and in a manner that embraces ethical, legal, and larger societal values. Evaluating emerging best practice and lessons learned from the above-mentioned initiatives will be insightful and the integration of responsible research and innovation practices into the funding process by the UK (as noted in section 7.2) may also provide a useful model for publicly funded research.

10.7. Implementation of regulatory frameworks

The greatest focus of regulatory frameworks which have implications for the governance of synthetic biology and which are relevant to the objectives

167 https://sci.hms.harvard.edu/.
168 https://ainowinstitute.org/.
169 https://www.editingnature.org/.
170 https://news.harvard.edu/gazette/story/2019/01/perspectives-on-gene-editing/.

of the Convention and its Protocols is the protection of people and the environment, while applying principles of risk assessment and risk management. Foremost, while the objectives are clearly different, it is evident that biosafety and biosecurity, at least in a containment context, are complementary regimes that benefit from an aligned approach. Indeed, in practice at the national level, these are often addressed together through a single biorisk management programme ensuring compliance with the requirements and good practices set out in international guidance documents and local legislative frameworks (Beeckman & Rüdelsheim, 2020). These risk assessment and management practices are embedded in a robust framework of international, regional and national regulations dealing predominantly with research, handling, release and standards that ensure the protection of human, animal and plant health as well as the environment.

The rapid advancement of the underlying science and the exponential rise in some potential applications of synthetic biology is, in many cases, challenging the speed at which national and international governance frameworks may need to, or can, adapt. This is especially critical when it comes to how synthetic biology tools, technologies and applications are/will be described by the various legal frameworks, which will then provide the relevant mandate to regulate (or not) activities with them. The challenge will be in arriving at international consensus with respect to definitions, including which product characteristics and/or technologies will fall under them. It is expected that challenges arising from differences in regulatory approaches for biotechnology (e.g. process-based versus product-based) will continue to be faced for those organisms resulting from synthetic biology.

The different fundamental objectives of the international trade and environmental regimes can lead to tensions in the regulatory measures taken to achieve these objectives. Strengthening the coherence of these two systems requires measures to be taken at national and supranational levels to ensure that they are implemented in a mutually supportive manner, and society will have a key role to play. A formal analysis of the trajectory or dynamics that the interpretative flexibility is taking may also be useful to anticipate the social perception of these decisions.

As the first synthetic biology applications have begun commercial deployment and others approach potential environmental release, they provide a useful lens through which to evaluate overlaps and potential gaps in the governance of synthetic biology, which require further alignment. Early cases give insights of the various regulatory challenges. As an example, the release of a disease vector LMO with an engineered gene drive will likely require assessment and approval by several national authorities, each referring to a particular objective. Authorities mandated to ensure conserving and protecting biodiversity will refer to the Convention and its Cartagena Protocol on Biosafety, while improving human health through the development of effective and safe health interventions may be inspired by WHO guidance. In assessing such an application, a field evaluation will likely be suggested as part of a stepwise approach. However, the purpose of these evaluations may be different depending upon the framework under which they will be undertaken. In this sense, the design of these field evaluations may differ in scope and approach, with one safeguarding the conservation and sustainable use of biological diversity, and the other attempting to demonstrate efficacy regarding its intended public health use.

Such diverging orientations could pose practical but not necessarily novel challenges in the design of field evaluations of LMOs containing engineered gene drives, especially when aiming to minimize risk while demonstrating positive health impacts. It shows that interaction and coordination among different regulatory agencies with overlapping and or complementary responsibilities will be required, and will be especially important to ensure that any identified gaps are filled. Any shortcomings in such an approach have the potential to be further perpetuated and exacerbated by the absence of integrated guidance provided under each regime or implementation under national law. WHO, for example, has published a guidance framework to establish best practices for research into genetically modified

mosquitoes which includes recommendations on biosafety, ethics, regulation, and efficacy, in addition to gene drive-specific recommendations (as noted in subsection 9.2.1(d)). On the other hand, under the Convention and its Protocols, discussions are ongoing regarding potential additional risk assessment guidance for LMOs containing engineered gene drives. A successful international strategy for engineered gene drive governance for vector mosquito control would benefit from streamlined communication and integrated guidance across international regimes, particularly the Convention and WHO (Kelsey et al., 2020).

Divergent and competing interests and views are another element that adds to the complexity of the implementation of regulatory frameworks. For instance, there is a view that humans should not intervene in nature at all using a technology such as gene drive-modified organisms. Reconciling this argument with proposals to use engineered gene drives to relieve the burden of infectious disease in humans, conserve species, or increase agricultural productivity is not straightforward, as either using this technology irresponsibly or not using it at all requiring a continuing reliance upon current chemical approaches could prove damaging to humans, our welfare, and our planet. Ultimately, reconciling such competing interests and values will determine how active society is willing to continue to be in shaping populations and ecosystems. It also highlights the importance of using a multidisciplinary or interdisciplinary approach for making decisions related to the development and application of engineered gene drive technology.

Given the diversity of applications and the vague boundaries between them as well as the combination of different types of rapidly evolving technologies, alignment between the different policy areas can be optimized through consideration on the appropriateness of a product-based or process-based approach to inform the risk assessment process. Whereas process and technical aspects are an integral and essential part of the case-specific risk assessment, addressing potential risks while promoting beneficial utilization of synthetic biology can be achieved when considering concrete applications and products. Ultimately, decisions to deploy synthetic biology organisms in the interests of human health, conservation, or increased agricultural productivity will invariably be a product of local power relationships, cultural traditions, and social norms, among other factors.

11. Conclusions

Since the publication of the previous Technical Series document (No. 82) on synthetic biology in 2015, the range of synthetic biology applications has continued to increase. With a broader array of applications under research, in development, or nearing environmental release and new options to contribute to addressing global challenges, this multidisciplinary area of research has garnered wider awareness and attracted much attention. Further, synthetic biology may also offer additional prospects for contributing to achieving diverse United Nations Sustainable Development Goals, including Zero Hunger; Good Health and Well-being; Clean Water and Sanitation; Affordable and Clean Energy; Industry, Innovation and Infrastructure; Responsible Consumption and Production; Climate Action; Life below Water; and Life on Land. However, this has also brought heightened awareness and recognition of the importance of synthetic biology's governance and regulation, since new challenges may also emerge in relation to the suitability of existing frameworks and how international policy should deal with synthetic biology. As the field continues to advance and more applications are envisioned, there is a growing pressure towards achieving governance clarity.

A comparison between the synthetic biology applications listed in Technical Series No. 82 and those in the present document indicates that the number that are commercially available or near-term stages has grown, which is consistent with the increasing activity and interest in synthetic biology. As was the case in 2015, applications for contained use still represent most applications commercially available. Some examples are semi-synthetic artemisinin, squalene, and vanillin, which were available in

2015 and continue to be available now. In contrast, a change since 2015 is the availability of commercial products for use directly in the environment, including genome-edited soya bean and engineered bacteria. Additional products intended for environmental release are under research, such as genetic control for conservation and control of vector-borne diseases. However, time has shown that the relationship between early research and development and the commercialization or deployment of products is not linear, and not every application that is under research will eventually progress to an advanced stage of environmental release, even if it is not commercial. These recent developments have compounded the need to continue acquiring additional data and knowledge to support the discussions about potential impacts from synthetic biology. There are divergent views on what the impacts could be, their magnitude or relevance, as well as how they should be assessed or managed. It is also noteworthy to consider that most regulatory mechanisms (including those discussed in the present document) were developed before some of the tools that enable synthetic biology and even the term itself became widely used. Therefore, they may not provide sufficient oversight, in terms of scope and scale, for some of the potential impacts from synthetic biology.

As has been shown by some of the examples (section 3) and discussions (section 5), the potential impacts from synthetic biology applications may be complex in nature and can cause challenges to their regulatory oversight. There is a strong call for robust and science-based risk assessments as well as technical expertise and knowledge needed to properly assess any potential impacts. The way these issues are being addressed continues to vary from country to country. Although existing legislation and instruments (such as the Cartagena Protocol on Biosafety through its Annex III on risk assessment) may provide a good basis for addressing some of these regulatory challenges, there is also a perceived need for the development of additional tools to complement this and other existing methodologies. Further, although not all applications of synthetic biology may require detection and identification, the current apparent inability to do so for specific applications adds complexity and may strain the abilities of developing nations whose regulatory frameworks may not be fully developed. However, it should be noted that implementation and capacity challenges are not unique to synthetic biology and are the subject of extensive discussion under the Convention and the Protocol.

By applying novel genetic techniques, synthetic biology offers the opportunity to address environmental challenges. However, actual benefits are as yet mostly unclear and the intentional or accidental release of products from synthetic biology into the environment could, depending on the circumstances, have significant, even detrimental impacts on biodiversity. To avoid unintended irreversible environmental damage and their associated geopolitical threats, innovative research guidelines, governance methods, integration with social sciences, and engagement with communities are needed. Capacity-building, information and knowledge-sharing, technology transfer, risk assessor training, and integration with academia will also play an important role. In addition, considering the fast pace of development of synthetic biology, and the challenge for some regulatory regimes to cope with potential new applications, an early screening of what is under research and development as well as those close to release and/or commercialization would be critical in providing timely information for countries and organizations to react and adapt if necessary.

Calls for improved governance of synthetic biology, including addressing gaps in the international legal and regulatory frameworks, place significant emphasis on the need to better address societal, economic, and ethical dimensions. Enhanced regulatory oversight addressing these dimensions appears desirable to promote public trust and acceptance; however, the international treaties, laws, processes and initiatives analysed appear not to have been designed with this foremost in mind.

A common feature of articles discussing the governance of synthetic biology is a focus on the operation of international regimes as silos and on the need to better integrate/coordinate such governance across

international fora. The need to expand the focus of the governance beyond human health and the environment to a more holistic approach that also encompasses social impact, ethical principles, and elements of social justice is also a focus, including the need for inclusive decision-making and community engagement in line with the approaches noted in section 10.6. Such approaches are suggested as a means of better equipping the international governance framework to enhance oversight of future field testing and public trust concerning emerging technologies. As such, they help inform modalities for participatory decision-making and are therefore particularly relevant in the context of FPIC of IPLCs under the Convention, which was recently been underscored in decision 14/19 of the Conference of the Parties.[171]

Community engagement is rooted in established ethical principles concerning prior informed consent concerning human research subjects (Resnik, 2018; Singh, 2019); however, challenges remain in the translation of principles under the Convention concerning the FPIC of IPLCs into effective and standardized protocols for community consultations and participatory decision-making. In the context of engineered gene drives which have the potential to affect environments bound by kinship, cultural identity and life-sustaining resources, it has been noted that "*it is not enough for the communities in those environments, including historically marginalized peoples, simply to be present at the debating table — their voices must be heard*" (Kofler, 2019). Further guidance on community engagement generally and specifically on FPIC appears a logical next step to be undertaken under the auspices of the Convention and other international fora focused on strengthening governance related to synthetic biology applications. Decision XIII/18 of the Conference of the Parties, for instance, notes the importance of IPLCs having the capacity and autonomy to develop, as appropriate, community protocols or processes for FPIC. Given the continuously evolving nature of the principles underpinning FPIC, such guidance may benefit from closer evaluation of approaches involving Responsible Research and Innovation, and Governance Coordinating Committees (noted in section 10.6) given their focus on principles of transparency, accountability, integrity, and capacity.

The overlaps and gaps identified in this update suggest that opportunities exist for increased coordination among the Convention and its Protocols, and for increased information exchange with other relevant international treaties, processes and initiatives converging on the governance of synthetic biology. Such multilateral coordination could minimize duplication and fragmentation and promote international governance of synthetic biology in a holistic and integrated manner. The Convention's experience in establishing for instance the Liaison Group of Biodiversity-related Conventions pursuant to decision VII/26 (paragraphs 1 and 2) and its implementation of a road map to optimize synergies could be instructive in this regard. Similarly, the Convention already has successful structures, guidelines and processes, such as the Collaborative Partnership on Sustainable Wildlife Management, that can be duplicated in the context of synthetic biology. This would provide a voluntary partnership of the international organizations with a substantive mandate and programmes on synthetic biology to address relevant issues that require a consolidated and coordinated approach. In addition, approaches already important for the Convention activities, such as the ecosystem approach and One Health, could also offer important information for actions related to synthetic biology.

The cross-cutting nature of synthetic biology, added to its broadness as a multidisciplinary area of research, are important factors to consider in any potential scenario towards its governance and regulation. The components, organisms and products resulting from synthetic biology will fall under the scope of several regulatory mechanisms. While some instruments are sufficiently broad to address some of the current issues related to synthetic biology, ambiguities still exist relating to the practical implementation of these instruments. Furthermore,

171 https://www.cbd.int/doc/decisions/cop-14/cop-14-dec-19-en.pdf.

it is unlikely that a single entity will have the necessary set of tools (e.g. mandate, capacity, knowledge, etc.) to have a meaningful impact on its own. Gaps in international governance could occur where components and products resulting from synthetic biology techniques do not fall within the scope of a treaty regime. In this sense, regulatory approaches which use general purpose criteria may facilitate adaptation regardless of future scientific and technological developments in synthetic biology. For example, the Biological Weapons Convention, which applies to all scientific and technological developments whose intended use is inconsistent with its objectives (as opposed to regulating specific biological and chemical agents) appears sufficiently flexible to apply to the use of components, organisms and products resulting from synthetic biology techniques for hostile purposes or in armed conflict, regardless of future scientific and technological developments.

Finally, as synthetic biology will continue to grow in relevance and importance due to the opportunities that it offers towards providing new tools and approaches for addressing global challenges, it is imperative that resources also be available for the development and/or adaptation of regulatory systems that could provide the needed safety that should accompany any potential use.

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