Secretariat of the
Convention on
Biological Diversity

CBD Technical Series No. 82

82

SYNTHETIC BIOLOGY

FOREWORD

To be added by SCBD at a later stage.

BACKGROUND

In decision X/13, the Conference of the Parties invited Parties, other Governments and relevant organizations to submit information on, *inter alia*, synthetic biology for consideration by the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA), in accordance with the procedures outlined in decision IX/29, while applying the precautionary approach to the field release of synthetic life, cell or genome into the environment.

Following the consideration of information on synthetic biology during the sixteenth meeting of the SBSTTA, the Conference of the Parties, in decision XI/11, noting the need to consider the potential positive and negative impacts of components, organisms and products resulting from synthetic biology techniques on the conservation and sustainable use of biodiversity, requested the Executive Secretary to invite the submission of additional relevant information on this matter in a compiled and synthesised manner. The Secretariat was also requested to consider possible gaps and overlaps with the applicable provisions of the Convention, its Protocols and other relevant agreements. A synthesis of this information was thus prepared, peer-reviewed and subsequently considered by the eighteenth meeting of the SBSTTA. The documents were then further revised on the basis of comments from the SBSTTA and peer review process, and submitted for consideration by the twelfth meeting of the Conference of the Parties to the Convention on Biological Diversity. The documents were then issued as CBD Technical Series on Synthetic Biology 82 edition in 2015.

In 2018, through decision 14/19 paragraph 17 (c) the COP requested the Executive Secretary to update the Technical Series on Synthetic Biology for consideration by the Subsidiary Body on Scientific, Technical and Technological Advice based on the peer review of scientific information and other relevant information; request that the present edition attempts to address.

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CONTENTS

1 ABBREVIATIONS AND ACRONYMS

3D	Three-dimension
A, C, G, T	Adenine, cytosine, guanine, thymine
ABNJ	Areas beyond national jurisdiction
ABS	Access and benefit-sharing
AHTEG	Ad Hoc Technical Expert Group
AHTEG-RARM	Ad Hoc Technical Expert Group on Risk Assessment and Risk Management
AIA	Advanced informed agreement
BBNJ	Biodiversity beyond national jurisdiction
BCH	Biosafety Clearing-House
bp	Basepair
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 the International trade in Endangered Species of Wild Fauna and Flora
COP	Conference of the Parties
CRISPR	Clustered regularly interspaced palindromic repeats
DARPA	Defense Advanced Research Projects Agency
DBTL	Design Build Test Learn
DIYbio	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
HEGAA	Horizontal environmental genetic alteration agent
IAG	Independent advisory group
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
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 organisation
nt	Nucleotide
ODM	Oligonucleotide-directed mutagenesis
OECD	Organization for Economic Co-operation and Development
OIE	World Organization for Animal Health
OPCW	
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
SDN	Site-directed nuclease
siRNA	Small interfering RNA
SPS	Sanitary and Phytosanitary Standards
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	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

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A. 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, 2015a). As the discipline continues to advance rapidly and expand beyond the confines of the laboratory, the potential of synthetic biology carries hopes and aspirations to address a multitude of global challenges related to food, health and the environment, among others, but also concerns about potential risks including those associated to biodiversity.

Synthetic biology relies on a suite of supporting technologies and tools, some of which are also used in genetic engineering. The emergence of several sophisticated technologies has greatly impacted the sector in the last years. As a consequence, the number of applications, especially those that make use of genome editing technology, has increased exponentially and has led to advances in plant and animal engineering, personalised medicine, and clinical therapeutics. Particularly, CRISPR-Cas technology is having impacts in agriculture, especially in increasing plant yield, quality, disease resistance and herbicide resistance, breeding, and accelerated domestication. Moreover, technologies such as engineered gene drives can now potentially be applied to a wide variety of organisms as a tool to spread traits throughout a population. These tools and areas of research have led to a range of synthetic biology applications that can be categorised by their intended use in: (i) contained, industrial, or laboratory settings, (ii) semi-managed, managed, or urban settings, or (iii) unmanaged or wild settings. Amongst each of these categories, several products are being commercialised, or are in a research and development stage.

Although synthetic biology is often referred to as a single discipline, the numerous areas of synthetic biology research represent a wide array of potential impacts, some of which are complex in nature and as a result, there is a continuing need to acquire further data and knowledge to support the discussions about potential impacts. The use of synthetic biology triggers a wide variety of views related to risks and benefits, moral and ethical values, along with broader issues such as socio-economic aspects. Therefore, a science-based assessment of any potential impact is seen as part of a wider decision-making activity; one that evaluates such economic, political, moral, and ethical concerns alongside a scientific analysis of the expected or potential changes that would result from using technology. It is also important to stress that due to the diverse nature of the potential impacts, they cannot be generalised for all synthetic biology applications, and they should, by necessity, be considered on a case-by-case basis. In this light, as synthetic biology applications approach commercial deployment and potential environmental release, this is bringing challenges to building consensus on how they are to be regulated, either under the same regimes as classical genetic engineering albeit with adaptations, or under new regimes yet to emerge. At a national and regional levels, regulatory frameworks are developing at different rates and with differing perspectives with respect to synthetic biology governance. At an international level, the range of actors potentially involved in synthetic biology governance has grown and is likely to continue to grow in light of the cross-cutting nature of the matter.

Currently, the governance of synthetic biology is supported by a range of international laws, processes, and initiatives, considering a number of factors including the products and processes involved, the purpose for which they are applied, and the transboundary implications of their use. However, this fragmented landscape at the international level, creates a complex scenario with the potential for regulatory gaps and areas of convergence to develop. 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 challenges that go beyond the scientific areas, and call to also consider societal, economic, and ethical dimensions. Enhanced regulatory oversight addressing these dimensions appears desirable to promote public trust and acceptance, however, the international laws, processes and initiatives analysed appear ill-equipped to address several of these dimensions. 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 impacts of synthetic biology and its regulation, particularly as they relate to biodiversity and biosafety.

There is a recognised need to first better integrate and coordinate governance of synthetic biology, and secondly, to expand the focus of governance beyond the focus on biosafety, human health and the environment to a more holistic approach that also encompasses social impact, ethical principles, and elements of social justice, in accordance with national circumstances. To avoid unintended irreversible environmental damage and associated geopolitical challenges, innovative research guidelines, governance methods, integration with social sciences, and engagement with communities are needed. As we think about advancing synthetic biology into the future, the challenge is integrating the scientific freedom that allows research and product development to move ahead while acting responsibly and in a manner that embraces ethical, legal, and larger societal values.

This document is an update of the CBD Technical Series No. 82 on Synthetic Biology (2015).

KEY MESSAGES

The current state of synthetic biology

1. **Synthetic biology is a cross-cutting and rapidly advancing discipline that has gained great attention due to its increasing relevance to the environment, food and health among other global challenges.**
   The international discussions about synthetic biology have come to the forefront and are now much more visible and have drawn attention from a wide range of actors. However, divergent views on what is considered "synthetic biology" pose challenges at various levels that range from the governance of the issue to more specific aspects such as challenges in applying transboundary movements requirements, for instance those of the Cartagena Protocol, due to the inability to identify some synthetic biology products, asynchronous authorisations on trade, amongst others. In addition, now that products of synthetic biology are commercially available or in advance stages of research and development, this is bringing challenges to building consensus on whether and how they are to be regulated, either under the same regimes (i.e. those used for classical genetic engineering) albeit with adaptations, or under new regimes. The current debate also echoes similar views expressed at the emergence of classical genetic engineering where developments were considered inherently risky by some, or not presenting any unique or novel risks by others. 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 on biodiversity-related issues as well as cultural, social, ethical and economic considerations.

2. **The potential of the synthetic biology toolbox is boundless, and so are the opportunities for synthetic biology to have an impact in an unprecedented manner.**
   Synthetic biology's rapid development can be seen in the numerous applications that have reached the market and those that are under research and development. Some of these applications directly target global challenges such as climate change by for instance aiming at increasing the resilience of species to climate change (i.e. in corals), or in designing "next generation" biofuels. Similarly, synthetic biology applications are also targeting the replacement of natural materials to take pressure of wild populations, as is the case of the production of recombinant Factor C (rFC) from synthetic horseshoe crab blood, synthetic rhinoceros horns and squalene, each of which could reduce or remove the need to exploit wild species. Applications containing engineered gene drives are being designed to modify, suppress or eradicate populations of various target species. The development of applications based on engineered gene drives targeting invasive alien species, human disease vectors and agricultural pests are currently under development. There are also a wide range of synthetic biology products that are currently on the market for various uses, such as such as semi-synthetic artemisinin, squalene, vanillin, shikimic acid, and select fragrances and flavours. These are only some of the many examples of synthetic biology applications that are having and could have an impact in an unprecedented manner.

3. **The value of the synthetic biology market has increased exponentially.**
While some research in synthetic biology is focused on promoting a greater understanding of the essential functions of genomes, most of the research is focused on commercial and industrial applications. The global synthetic biology market was estimated to be valued at USD 6.8 billion in 2020 and is projected to grow at a compound annual growth rate of 23.9 % during the period of 2020 - 2025. This can be attributed to the rising demand for products, 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 CRISPR-toolbox.

4. **Supporting technologies and tools have rapidly evolved, spawning even more types and numbers of applications, to the extent that synthetic biology is essentially ubiquitous in life science**
Synthetic biology relies on a suite of supporting technologies and tools that enable the engineering and creation of biological components. These tools could include DNA synthesis, directed evolution, genome editing, engineered gene drives, RNA interference, 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 complex and rich scenario for developing new applications and products. However, the pace of development of some of these disciplines is not uniform and as such, the degree of maturity of plant synthetic biology is lagging bacterial, yeast and mammalian systems, where these approaches are already reshaping fundamental research and the biotechnology or biopharmaceutics industries.

5. **Despite its potentially global deployment, research and development in synthetic biology mostly occurs in a limited number of countries**
By 2017, more than 25,000 authors at 3700 organisations located in 79 countries had contributed to the synthetic biology research. Since 1980, 13050 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. There were a reported 2800 sponsors of such global research. Despite the global spread of research sponsors, the authors reported a concentration amongst a subset of funding agencies. A cluster of top 20 global sponsors of synthetic biology research were recognised in 70.6% of the synthetic biology articles. This cluster included six funders from the USA, three from China, two for Canada, Germany, Japan, the UK, and the EU each one, and one from South Korea. The great majority of funders in the top 50 are public research councils or government agencies.

Potential impacts from synthetic biology

6. **Synthetic biology products designed for use in managed, semi-managed and urban situations attract the greatest attention as those are the ones that the public at large will have greatest interactions with.**
Synthetic biology has provided an unprecedented toolbox for tailoring organisms for new applications and products. 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 organism is changing rapidly. Currently, of those synthetic biology products that are commercially available and intended for use in semi-managed, managed, or urban settings, there are two genome edited crops, self-limiting insects, and biological nitrogen fertiliser based on engineered bacteria It is expected that some other genome edited organisms and potentially those containing engineered gene drives could reach the market in a few years. On the other hand, synthetic biology products intended for unmanaged or wild settings remain in an early stage of research. Table 1 provides a summary of the synthetic biology applications categorised by their intended use and their level of development. Synthetic biology is therefore moving out of the laboratory environment towards other settings where interaction between people and biodiversity with these applications will be more common.

7. **As only a few synthetic biology applications developed for direct use in the environment have been commercialised, relatively little "real world" data has been collected concerning their potential impacts.**

The range of potential impacts of synthetic biology applications on the conservation and sustainable use of biodiversity remains largely hypothetical/speculative due to the limited number of commercial products developed specifically for use in the environment that are currently available. Thus, the discussions on potential impacts have been informed mostly by previous experience with LMOs and associated concerns. This is bringing challenges to arriving at consensus on whether synthetic biology applications are to be assessed, and regulated under the same regime, which itself is beginning to adapt to these applications.

8. **Many of the impacts that were originally expected were overly simplistic in nature, with latest experience demonstrating that the situation is far more nuanced and with multiple factors adding to the complexity.**

For example, the replacement of natural products with products resulting from synthetic biology could lessen the pressure on natural habitats but could also disrupt *in situ* conservation projects. There may be the 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, but they could also be mixed with (illegal) natural specimens. The displacement of some of the natural products (i.e. naturally occurring molecules obtained from plants) can also potentially ease negative pressures on wild or cultivated species, but it can also displace cultivation practices, often in topical and sub-tropical 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. Similar situations and examples could be cited for other synthetic biology applications. This complex web of potential interactions derived from the use of synthetic biology applications in various scenarios is therefore adding to the challenges of assessing the potential impacts that could be associated with their use.

Communication, engagement and transparency

9. **Early community engagement in synthetic biology research is key for transparency.**

Recognising the global nature of synthetic biology applications and the fact that local communities are most likely to be impacted first, 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 IPLCs could provide opportunities to better inform the public and potentially impacted communities and allow for discussions related to potential benefits, potential risks, and concerns. Sufficiently broad and inclusive discussions could in turn improve public trust through the development of safety measures and policies, 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 communities will be important when considering deployment in other geographical locations, especially as there may be a need for further building of local regulatory capabilities.

10. **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 characterised 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 and should instead be informed through consultation with a broad set of stakeholders, including the populations likely to be impacted most. For emerging technologies, especially synthetic biology, that 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, research into areas of

uncertainty, and the development and testing of biosafety features. It would also allow any necessary adaptation of regulations and conventions in light of emerging information on benefits, risks, policy gaps, and, 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 recognised, particularly related to the free prior informed consent (FPIC) of Indigenous Peoples and Local Communities (IPLCs) concerning the environmental release of engineered gene drives.

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

"Dual use" concerns raised by synthetic biology, whereby research with legitimate scientific purpose may be misused to pose a biologic threat to public health and/or national security, threatens to undermine public confidence and must be appropriately addressed. The "DIY Bio" community in particular has raised concerns regarding oversight and containment. They have been proactive about biosecurity and biosecurity education, but concerns persist as to what other 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 that the research was 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.
of those countries form the basis of discussions aimed at reaching a consensus at the international level.

Synthetic biology regulation and governance

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

Due to its interdisciplinary nature, synthetic biology presents particular challenges for the regulatory system as applications of synthetic biology have the potential to accelerate the pace of technological development across multiple sectors, with the promise of helping to solve some of humanities greatest challenges this century. To harness such benefits, much will depend upon whether an enabling policy and regulatory environment is in place to maximise potential benefits while minimizing any potential risks. Often, international and national regulatory regimes tend to focus on biosafety risks rather than a more holistic approach that takes into account a range of public interest issues related to the biosecurity, ethics, societal, cultural and economic implications of synthetic biology more broadly, as well as potential benefits related to biodiversity conservation and sustainable use. In this sense, a new paradigm for regulating synthetic biology applications is needed that looks beyond just biosafety.

13. Better understanding on what to expect in terms of developments will play a key role in helping regulatory systems to cope-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 commercialisation perspectives will be critical in providing timely information for countries and organisations to react and adapt if necessary.

14. Most regulatory mechanisms were developed before the term synthetic biology became widely used and may need updating to address synthetic biology.

Synthetic biology is a new discipline that combines many technical areas, tools and approaches, therefore most of these regulatory mechanisms were not developed with the necessary scope and scale

that some of the potential impacts of synthetic biology may present. This is a challenge not only under the Convention and its Protocols but for other regulatory frameworks.

The developments in synthetic biology will put even greater pressure on those developing countries that have not yet developed national biosafety frameworks for conventional LMOs. These systems will have to develop/evolve to already include some of these complex elements. This will require a concerted effort from all stakeholders to adapt existing frameworks in order to "future-proof" them for synthetic biology applications.

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

Considering the broad scope of not only synthetic biology research, but also the potential impacts of its products and applications, it is not surprising that no international treaty framework nor institutions exist with a sufficient mandate to regulate the full spectrum of possible synthetic biology activities or impacts. A fragmented landscape of international instruments, regimes and initiatives have 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 amongst others. As 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 CBD provides unique opportunities for hosting discussions aimed at improving coordination and addressing challenges and cooperation opportunities which are apparent in the governance of synthetic biology without the need to invent/create another series of fora. In addition, the cross-cutting nature of synthetic biology added to its broadness as a discipline, are important factors to consider in any potential scenario towards its governance and regulation. 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. Therefore, aspects such as coordination, cooperation, capacity-building, knowledge-sharing and communication are of paramount importance. The governance of synthetic biology cannot advance 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, potential regulation and potential use.

16. **National and regional regulatory frameworks are developing at different rates and with differing perspectives with respect to synthetic biology governance.**

This fragmented landscape creates a complex scenario at the international level, with the potential for regulatory gaps and areas of convergence to develop. This is further exacerbated by the large number of near-market applications, and as such, there is a growing urgency to discuss the evolution of a more cohesive international regulatory environment. Moreover, as synthetic biology will continue to grow in relevance and importance due to the opportunities that it offers towards solving global challenges, it is imperative that resources are available concurrently for research and development, and for the development and or adaptation of regulatory systems that could provide the needed safety that should accompany any potential use.

Table 1. Applications of synthetic biology, categorised by their intended use and degree of development.

Intended use	Research	Advanced development	Commercially available
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	• Cyanobacteria production platforms in contained environmental facilities. • Bio-fabricated wildlife products.	• Biopharma. • Carbon recycling. • Fabric. • Cosmetic and fragrances. • Food and food ingredients.

	for basic research and production of pharmaceuticals. • Re-creation of an extinct infectious horsepox virus from chemically synthesised DNA fragments. • Genetically engineered bio-containment systems within the cell. • Digital information storage using DNA molecules. • Synthetic biosensing circuits and biosensors.	• Cultured leather products. • Plant-based vaccines • Engineered phages as anti-microbials. • Engineered probiotics for the production and in vivo delivery of medicines.	• Part, devices, and systems.
Semi-managed, managed, or urban settings	• Genetically engineered nitrogen-fixing bacteria and other genetically engineered bacteria for agriculture. • Insect delivery of modified viruses for the modification of crops (horizontal environmental genetic alteration agents [HEGAAs]) for biodefence and agriculture. • 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.	• Genome edited crop plants and farm animals. • Engineered gene drives in mosquitoes for control of vector-borne diseases. • Engineered gene drive for an agricultural pest. • Genetically engineered bio-containment systems in mosquitoes. • 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 and oilseed rape. • Self-limiting insects. • Biological nitrogen fertiliser based on engineered bacteria.
Unmanaged or wild settings	• Genetically engineered bacteria for environmental applications, such as	• No information	• No information

	bioremediation, biodegradation and biomining. • Conservation purposes and control of vector-borne disease. • Improving the resilience of wild animal and plant populations

B. 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.*" 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 (Secretariat of the Convention on Biological Diversity, 2000).

While there is no internationally agreed definition of "synthetic biology", in decision XIII/17, paragraph 4, the Conference of the Parties (COP) acknowledges that the outcome of the work of the Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology on the operational definition is "*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*", and considered it useful as a starting point for the purpose of facilitating scientific and technical deliberations under the Convention and its Protocols.

Acknowledging that there are different views concerning which techniques fall under the above definition of synthetic biology, the authors recognise that some of the processes or products described in this document may not be considered as synthetic biology approaches and applications by all readers, however the broadest interpretation has been made in order to be as inclusive as possible whilst at the same time not championing this interpretation as being definitive. 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 sector as a whole.

To remain true to decision 14/19, whenever possible the original structure and outline 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 82 version were also condensed or removed if now out-dated or surpassed by more recent developments or information.

Understanding that there had been an explosion of activity that had occurred under the auspices 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 24th meeting of the Subsidiary Body

on Scientific, Technical and Technological Advice (SBSTTA)[1]. This list of references itself was then updated during the last quarter of 2020 and 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, business and industry in print and electronic formats. Of primacy amongst the latter were documents from the Conference of the Parties and Conference of the Parties serving as the Meeting of the Parties of the Cartagena Protocol (MOP), such as reports of the Ad Hoc Technical Expert Groups (AHTEGs) and relevant decisions, as well as publications, guidance and opinions from regulatory authorities. Together, these were supplemented with information from websites and publications of other organisations, especially those entities mandated to implement the international laws, processes and initiatives listed in Section 9 and those civil society organisations 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 where mainly sourced from AHTEG reports and other CBD 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 synthetic biology sector as a whole. The authors have therefore attempted to exemplify the discussed topics with actual examples, but this was not always possible, and therefore, sections of more generalised text have resulted.

The update of the 82 version of the document has therefore been made on the basis of the above principles and bibliographic searches. In addition, Section C (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 commercialisation, and is also anchored on the rationale used by the Synthetic Biology AHTEG in its report from 2019 (SCBD, 2019). For Section D (Potential impacts of on the Conservation and Sustainable Use of Biological Diversity) the update was made considering that impacts cannot be generalised, and therefore, they should be considered on a case-by-case basis and linked to specific applications. In this light, the section was drafted considering specific examples of synthetic biology applications whenever possible. In doing so, the authors prioritised examples from applications that are either in advance states of research, are commercialised, or that have received significant international attention either through publications and literature, or through discussions at international fora (i.e. CBD and other international meetings). It is therefore important to note that this section is not meant to be an exhaustive list of potential impacts for every application of synthetic biology. The update of this 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 socio-economic and cultural considerations. Section E (Synthetic Biology Governance and Regulatory Perspectives) was updated acknowledging the many international organisations and initiatives, including the CBD 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 organisations 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 prioritised the inclusion of information on international initiatives and did not consider those of a more regional or national nature.

C. TECHNICAL BACKGROUND ON SYNTHETIC BIOLOGY

1. Supporting Technologies and Tools.

Synthetic biology relies on a suite of supporting technologies and tools, which are also used in genetic engineering, that have become dramatically faster and less expensive since the 1990s (El Karoui et al.,

[1] https://www.cbd.int/doc/c/b006/4abe/2f4e0cdaca9f3884c9b92607/sbstta-24-inf-06-en.pdf.

2019; The Royal Academy of Engineering, 2009). Computational modelling and the connected fields of bioinformatics and information sciences have catalysed synthetic biology research by making simulation possible and *in silico* testing of biological systems (Esvelt & Wang, 2013). 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 methods and computer programmes make it possible to read longer lengths of DNA at much faster speeds for less money, often by aligning short sequences of overlapping stretches of DNA through computer analysis (RAE, 2009).

By 2017, more than 25,000 authors at 3700 organisations located in 79 countries had contributed to the synthetic biology research (Shapira et al., 2017). Since 1980, 13050 papers on synthetic biology have been published; with the USA, UK, Germany, China, and France leading the number of publications (K. E. French, 2019; Shapira et al., 2017). 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 amongst a subset of funding agencies. A cluster of top 20 global sponsors of synthetic biology research were recognised in 70.6% of the synthetic biology articles. This cluster included six funders from the USA, three from China, two for Canada, Germany, Japan, the UK, and the EU each one, and one from South Korea. The great majority of funders in the top 50 are public research councils or government agencies (Shapira et al., 2017). Fuelled by the raising R&D activities, the synthetic biology market has experienced significant growth in the past decade (Meng & Ellis, 2020). The global synthetic biology market is estimated to be valued at USD 6.8 billion in 2020 and is projected to grow at a Compound Annual Growth Rate of 23.9% during the period of 2020 to 2025[2].

Similar to the divergent views on what is considered synthetic biology, there could also be different views on what could be considered a supporting technology or tool. This section provides information on some of the more widely used tools but is not meant to be an exhaustive list.

1.1. Chemical synthesis of DNA.

The ability to chemically synthesise DNA dates to the early 1970s when a 77 base pair (bp) double-stranded DNA was successfully synthesised (Wang et al., 2018). The introduction of automated DNA synthesis machines has saved time and effort on the part of researchers using synthesised DNA for experiments (Garfinkel & Friedman, 2010; Schmidt, 2009). Using proprietary techniques, machines can also create DNA strands up to the size of a gene, hundreds, or thousands of base pairs in length. Techniques for DNA assembly have also advanced, with laboratories having developed various *in vivo* assembly systems by which 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).

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 synthetic 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 synthetised, but for fragments ranging 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 startup companies are now pursuing the potential of enzymatic synthesis as a faster, even within a single

[2] MarketsandMarkets™ report elaborated by Area Science Park, Italy (22 February 2021).

day, and more efficient route to synthesise longer DNA sequences than is possible with traditional chemical means. Moreover, it is expected that in two or three years, the first benchtop DNA printer will be commercially available (Eisenstein, 2020).

1.2. Directed evolution.

Directed evolution is a supporting biotechnology method often employed for synthetic biology, consisting of iterative rounds of mutagenesis and screening or selection on the genome scale (V. 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. 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 direct genome evolution, the tools used are mainly related to growth conditions and tolerance to chemicals. For targeted directed genome evolution, 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.

1.3. Genome editing.

Synthetic biology also employs techniques for genome editing based on oligonucleotide-directed mutagenesis (ODM) and variants of site-directed nuclease (SDN) technologies. Chemically synthesised 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 mammal cells (Aarts & te Riele, 2010; Strouse et al., 2015).

SDN techniques include meganucleases (MN), zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and CRISPR. These techniques 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) or directed sequence changes (SDN-2 and ODM) at specific, predefined genomic loci. These approaches do not necessarily require the stable 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 supposed to be 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. 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 and are heritable (Eckerstorfer, Heissenberger, 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*, (T. Li et al., 2012) or a soya bean with improved oil quality (Haun et al., 2014). TALENs are recognised for their high degree of precision and control. Unlike CRISPR-Cas that relies on DNA-RNA interaction as the mechanism of target DNA recognition, TALEN relies in DNA-protein interactions (Gaj et al., 2016; Khalil, 2020).

An enormous increase of studies performing genome editing in crops has been reported, especially the CRISPR-Cas and related systems (e.g., base editing) which have been used lately for almost all studies and a rising number of market-oriented traits have been addressed by genome editing. In less than a decade, the number of applications of this powerful technology (CRISPR-Cas) has increased exponentially worldwide and has led to advances in plant and animal engineering, personalised medicine and clinical therapeutics.

Notably, the 2020 Nobel Prize in Chemistry was awarded to developers of this tool (Uyhazi & Bennett, 2021). It is expected that the advances in CRISPR-toolbox register a Compound Annual Growth Rate of 18% in the period 2021-2025[3].

CRISPR-Cas technology is having impacts in agriculture, especially in increasing plant yield, quality, disease resistance and herbicide resistance, breeding and accelerated domestication (Zhu et al., 2020). For instance, it is now possible to 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 breed plants to adapt them for urban agriculture uses (Kwon et al., 2020). CRISPR tools can also facilitate the precise control of plant chromosomal recombination (Taagen et al., 2020), thereby unlocking otherwise inaccessible genetic diversity. Next generation of CRISPR-Cas technologies and applications are already underway (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 since 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 (H. A. Rees & Liu, 2018). This technique precisely 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).

1.4. Engineered gene drives.

A gene drive is a phenomenon in which selfish genetic elements circumvent Mendel's laws of independent assortment and favour their own inheritance (Burt & Crisanti, 2018). Transposable elements, meiotic drive genes, homing endonuclease genes and *Wolbachia*, are examples of natural gene drive systems that spread at the expense of their hosts (Sinkins & Gould, 2006). The potential application of these natural gene drive systems to suppress populations 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. Different mechanisms have been developed, but essentially engineered gene drives are genetic elements that are inherited more frequently than expected based on Mendel's laws 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 mechanism that increases the frequency of their inheritance (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 can potentially be applied to a wide variety of organisms (Rode et al., 2020). These CRISPR gene drives are efficient tools to spread traits through a population: an individual carrying a CRISPR gene drive will produce offspring that all carry the gene drive (Patrick Rüdelsheim & Smets, 2018).

Amongst the various CRISPR gene drives being developed (homing, split homing, translocation, X-shredder, killer-rescue, cleave-and-rescue and toxin-antidote recessive embryo gene drives), CRISPR-based homing gene drives are the most adaptable to new species and populations and the most advanced in terms of technological development (Raban et al., 2020). They involve a piece of DNA that includes a guide

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

RNA (gRNA) gene and a cas9 gene (encoding the Cas9 endonuclease). The gRNA is designed to recognise 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 rapidly spread within the target population (Rode et al., 2020).

1.5. RNA-based tools.

RNA interference (RNAi) is an intrinsic cellular mechanism present 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)[4] 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 poly-adenylated tail, blocking ribosomal protein synthesis and/or epigenetic transcriptional repression. Naturally, RNAi protects cells from double stranded RNA (dsRNA) viruses, supresses 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 anti-sense, 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 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 or avoid food waste (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 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 datasets have facilitated the design of more specific and efficient dsRNA molecules with potentially fewer or negligible 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 utilised 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 – see below) have been integrated into engineered biological systems for applications spanning biosynthesis, metabolic engineering, bioremediation, to health and medicine (Jang et al., 2018; Liang et al., 2011). Similarly, natural, and engineered miRNAs have also been applied to modulate and 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*,

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

and riboswitches[5], 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).

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 titter 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 utilised 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., 2020; G. 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 (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 utilises 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).

1.6. Artificial Intelligence and Machine Learning.

Many engineering endeavours involve Design-Build-Test-Learn cycles to achieve optimal solutions (Figure 1). 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 arises as an effective artificial intelligence 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 datasets, which are utilized to construct mathematical models (training) to identify underlying regularities or patterns, which then can provide general predictions on unseen datasets without a need to understand the detailed biological mechanisms (Radivojević et al., 2020). Machine learning has been used to, e.g., predict pathway dynamics (Costello and Martin, 2018), predict DNA and RNA protein-binding sequences (Alipanahi et al., 2015), drug side effects (Shaked et al., 2016) or directed protein evolution/engineering (Wu et al., 2019; Yang et al., 2019).

[5] 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 Vézina Bédard et al. 2020).

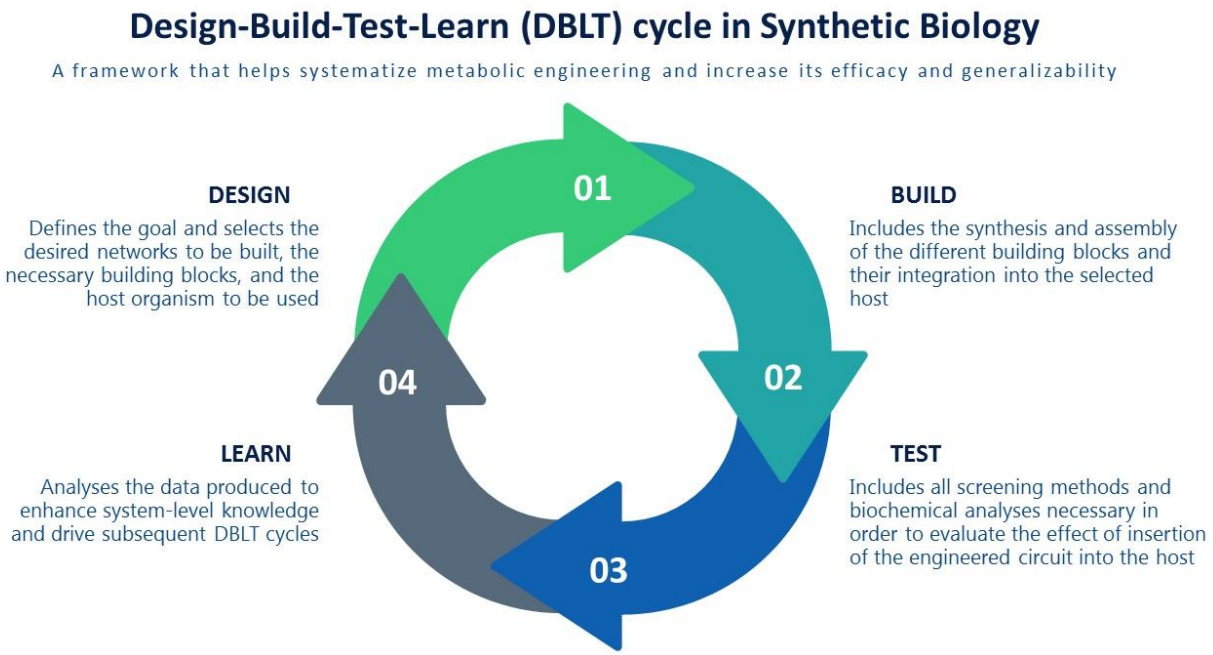

Figure 1. Iterative Design-Build-Test-Learn biological engineering cycles allow researchers to test large-scale genetic designs to enhance the process.

1.7. 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 industrialised, 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/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 the place where synthetic biology and artificial intelligence are converging into technology platforms with the capacity to create synthetic organisms and materials at a massive scale (Dixon et al., 2020). Many biofoundries are being built and a Global Biofoundry Alliance, consisting in 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 testing platforms (Crone et al., 2020) which can be quickly replicated around the world and increase capacity for testing and drug development.

1.8. BioBricks.

The foundational *Nature* paper by Endy (2005) applied three ideas from engineering to biology: standardisation 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. 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 precise 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 competition (iGEM). The iGEM Foundation (which runs the competition) also hosts an open website, the Registry of Standard Biological Parts[6] 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 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.

The areas of research that are considered synthetic biology[7] 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 synthetic biology 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 (M. Heinemann & Panke, 2006; Lam et al., 2009).

The biological concept of predictable and programmable genetic function can be traced to Jacob and Monod's Nobel prize-winning work on the *lac* operon in bacteria. 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 synthetic regulatory networks with predictable function. Another 40 years 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 unravel, as over the last decades numerous regulatory RNAs were have been discovered. For instance, sRNA regulators modulate protein expression through base pairing,

[6] https://igem.org/Registry

[7] Other areas of research sometimes included within synthetic biology include engineered synthetic multicellularity and the design of microbial consortia that communicate across species and coordinate towards human-specified ends (Lam et al., 2009; Maharbiz, 2012). These areas are not discussed in this document because they are not frequently included when synthetic biology is discussed, and commentators have not addressed them in terms of their implications for ethics, biosafety, biosecurity, or other aspects.

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 standardised, composable, and orthogonal parts, which can be scaled and tuned at will (Peters et al., 2015). Consequently, RNA regulators are effective tools to reprogram 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 many weeks or months of 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-characterised 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 e.g., a three-cell bacterial circuit based on an ecological strategy (Liao et al., 2019), or a mammalian macrophage-fibroblast circuit (X. 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 utilisation 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). 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 (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 optimisation of *Bacillus thuringiensis* toxin (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). As with DNA and RNA, synthetic circuits can be also based on proteins. Circuits composed of interacting proteins can be used to bypass gene regulation, interfacing directly with cellular pathways without genome modification. 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 (M.-R. 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 optimised in a broad design space of protein sequences and buffer compositions. In this context, artificial intelligence, and especially machine learning, have great potential to accelerate and improve the optimisation of protein properties, increasing their activity and safety as well as decreasing their development time and manufacturing costs (Narayanan et al., 2021). For instance, a unified rational protein engineering with sequence-based deep representation learning (Alley et al., 2019) and an improved protein structure prediction using potentials from deep learning (Senior et al., 2020) have recently been announced.

2.3. Metabolic pathway engineering.

While synthetic biology provides the components and information about different biological phenomena, metabolic engineering tries to apply all of this information towards the optimisation of the biological synthesis trajectory of the production of high-value-added biochemicals (García-Granados et al., 2019). Synthetic biology tools make it possible to build non-natural pathways that would be difficult to produce with classic genetic engineering techniques. Many of the first-wave synthetic biology commercial applications use metabolic pathway engineering to replicate naturally occurring molecules (Dasgupta et al., 2020). The majority of the current commercially available synthetic biology products listed in Section 3 falls 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 which are too complex to be chemically synthesised 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). Recently, such engineering has produced desired amounts of natural and non-natural chemicals as well as necessary materials (Dasgupta et al., 2020). 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 rewiring and lab evolution to convert *E. coli* into autotrophs, highlights the plasticity of bacterial metabolisms 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 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). For instance, South et al. (2019) investigated photorespiratory bypass strategies in transgenic tobacco plants to improve photosynthesis. In field trials, these transgenic tobacco plants were ~40 % 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 makes it also attractive to environmental scientists to use these

approaches for bioremediation of environmental contaminants through microorganisms (see Section 3.1.3) (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 optimise efforts of metabolic engineers (K. R. 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; Luo et al., 2018; Wang et al., 2009)[8]. 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 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; RAE, 2009; Glass et al., 2017; 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 38 % 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 synthesised DNA; it is also referred to as "synthetic genomics" (Konig et al., 2013). Thus far, researchers have reproduced the viral genomes of polio (Cello, 2002), the 1918 Spanish influenza (Basler et al., 2001; Tumpey, 2005) and the horsepox virus (Noyce et al., 2018). 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 pair 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 first synthetic chromosome of *Saccharomyces cerevisiae* synthesised in 2014 (Annaluru et al., 2014), the goal of the Sc2.0 initiative (Richardson et al., 2017) is to synthesise the first eukaryotic genome, the 12-Mb *S. cerevisiae* genome, is nearing completion. Similarly, the Genome Project-Write (GP-Write) has been proposed to engineer higher eukaryotes with gigabase-sized genomes (Boeke et al., 2016).

Minimisation 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 (L. 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 3-D genome organisation and karyotype engineering. The development of 3-D genome engineering tools, such as editing of structural DNA motifs, structural proteins or manipulating DNA looping, substantially facilitates our understanding of genome organisation principles and the causal relationship between 3-D 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 (Z. Luo et al., 2018), instead of the normal sixteen chromosomes, without affecting the total number of genes, the transcriptome or growth.

[8] This section and the next on protocells are sometimes categorised together, and sometimes top-down and bottom-up genomic engineering are separated, but all are commonly included within the scope of synthetic biology.

2.5. Protocell or minimal cell construction.

Similar 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 new way for 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 vibrant, 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).

The Build-a-Cell Research Collaboration Network[9] is facilitating studies on 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 cell from non-living components. This network provides a formalised structure for collaboration between individual labs, bridging geographical and disciplinary divides.

2.6. Xenobiology.

Xenobiology is the study of unusual life forms, based on biochemistry, that are not found in nature (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 xeno-nucleic 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; Vitor B 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 synthesise 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 is the first organism to stably propagate an expanded genetic code (Malyshev et al., 2014). The first stable semisynthetic 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 (hachi-)

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

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 bar-coding 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 2014, a bacterium was produced where one base pair of the original DNA was altered to XNA resulting is the first organism to stably propagate an expanded genetic code (Malyshev et al., 2014). In a recent development, an unnatural amino acid (UAA) has been genetically encoded at the defined site of the antibiotic resistance gene-encoded protein in *E. coli*. When UAAs are not in the culture medium, there was no expression in the antibiotic resistance gene-encoded protein. Thus, the site-specific incorporation UAA mutagenesis system could be used to control and expand the use of conditional selectable marker, and the technique is used to facilitate a rapid continuous genome editing in the bacterium (X. Xu et al., 2020). On the other hand, the first demonstration of enforcing an expanded genetic code to incorporate 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 holds 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 will be highly desirable in the chemical industry (Acevedo-Rocha & Budisa, 2016). At the same time, an organism that is alienated from existing ones and relies on a certain man-made cocktail of chemicals, 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) 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, characterise, and interrogate synthetic biological circuits. The cell-free transcription–translation platform, known as *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 characterisation of CRISPR elements or construction of synthetic cells. Synthetic RNA circuits are also

efficiently and easily characterised 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 synthetic biology have provided an unprecedented toolbox for tailoring organisms for new applications (Vickers & Small, 2018) and so, this section uses specific examples to demonstrate the incredibly large and diverse range of products that are being developed. The products described below reflect prominent examples and should not be considered as an exhaustive list. Further, they have been categorised 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, the products that are commercially available, in advanced development (e.g., products and applications that have been tested in confined field trials), and in research (e.g., exploratory research, proof of concepts) are specified.

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. Advanced development.

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

3.1.3. Research.

(a) *Genetically engineered bacteria for 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 compartmentalisation of external phosphate pollutants. Tay et al. (2017) developed a biosensor *E. coli* strain capable of both simultaneously sensing mercury and producing a mercury-absorbing, extracellular protein nanofibers, which form a large surface area for mercury absorption. French et al. (2020) created overexpression vectors for *E. coli*, to engineer specific metabolic hydrocarbon pathway, towards the biodegradation of oil spill and, simultaneously, for horizontal gene transfer to indigenous bacterial populations for prolonged soil remediation. Additional examples, especially of xenobiotic clean-up 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 a synthetic biology approach, which could be easily adopted and generalised 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 (Urbina et al., 2019) that utilises metal-binding peptides in fungal mycelia to enhance metal recovery from aqueous solutions such as those found in bioremediation or biomining processes. Other examples of biological adsorption/chelation for biomining where synthetic biology could have an impact are reviewed by Capeness and Horsfall (2020).

(b) *Synthetic biology applications for conservation purposes and control of vector-borne disease.*

There are ongoing efforts to advance engineered gene drive research to scale up efforts to protect island communities and prevent island species extinctions (e.g., Genetic Biocontrol of Invasive Rodents programme). The use of engineered gene drive technology to control or eradicate invasive rodent populations on islands is being explored. 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). Genome editing tools are being used at Texas A&M University to create mice that are unable to bear female offspring (Piaggio et al., 2017). More recently, an engineered gene drive based on CRISPR-Cas9 was developed for mice (Grunwald et al., 2019).

Likewise, an ecological engineering project is currently underway that aims to prevent the spread of tick-borne Lyme disease by using CRISPR-based genome editing to heritably immunise 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 normal heritance of a CRISPR-based modification.

(c) *Improving the resilience of wild animal and plant populations.*

Synthetic biology is currently being applied to conservation (Piaggio et al., 2017). In ocean ecosystems, for instance, novel genetic rescue tools have been reported (B. J. Novak et al., 2020), especially for corals and kelp. The first genome-edited coral (Cleves et al., 2018) has led to the idea of using genetic modification to increase the resilience of threatened species against anthropogenic climate change. 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 (Coleman & Goold, 2019), either by direct manipulation of kelp genomes or indirectly through the engineering of kelp-interacting stressor communities.

Terrestrial organisms are also being subjected to research. The animal model *Xenopus laevis* (the African clawed frog) has been genetically engineered with CRISPR technology to alter its immune system (Banach et al., 2017), a development which may enable resistance to specific amphibian pathogens. For over thirty years, a coordinated recovery effort has been underway for the Black-footed Ferret, one of the most endangered species in the USA[10] (Wisely et al., 2015). The team announced that the world's first successfully cloned Black-footed Ferret was born on 10 December 2020[11]. It is a clone of a wild-caught Black-footed Ferret whose cell line was cryopreserved in 1988, the genome of which possesses more genetic diversity than the current Black-footed Ferret population. In addition to cloning, researchers have also proposed the application of genome editing techniques to introduce plague resistance (B. J. Novak et al., 2018).

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

3.2.1. Commercially available.

(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. (Haun et al., 2014) Commercial production began in 2019.

(b) *Biological nitrogen fertiliser for maize based on engineered bacteria (Pivot Bio).*

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

(c) *Genome edited oilseed rape tolerant to herbicides (Cibus).*

Using Cibus' Rapid Trait Development System™ (ODM), *Brassica napus* acetohydroxyacid synthase was mutated to confer tolerance to imidazolinone herbicides (Cibus, 2014; Schopke et al., 2008).

(d) *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)

3.2.2. Advanced development.

(a) *Genome edited plants and animals.*

Menz et al. (2020) have recently reviewed and estimated that 140 genome-edited cultivars of 36 crops that improve yields, nutrition, and pest resistance, 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 in 2021 (Voigt, 2020). Animals are also being subjected to CRISPR-based genome editing. Voigt (2020), Brandt and Barrangou (2019), and Bishop and Van Eenennaam (2020) reviewed and reported 67 animal examples that are being developed by genome editing, including hornless

[10] https://reviverestore.org/projects/black-footed-ferret/

[11] https://www.fws.gov/mountain-prairie/pressrel/2021/02182021-USFWS-and-Partners-Innovative-Genetic-Cloning-Research-Black-footed-Ferret-Conservation.php.

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 be a better host for human organs, with preclinical trials in 2020 (Voigt, 2020), which could alleviate a global shortage of transplant organs (Servick, 2019).

(b) *Engineered gene drives in mosquito for potential control of vector-borne diseases.*

Engineered gene drive organisms are intended to spread a desired trait into a population. Usually, they harbour at least two linked sets of genetic modifications: the new trait and the ability to drive the trait into a wild population (Simoni et al., 2020). Some engineered gene drive developments are in an advanced stage of development. Target Malaria[12] is a vector control international research alliance dedicated to supressing populations of *Anopheles* mosquitoes as a measure to reduce malaria incidences in Africa. Similar initiatives are also underway but in contained conditions. For instance, the University of California Irvine Malaria Initiative[13] is testing an engineered gene drive technology not to supress *Anopheles* populations, but instead to prevent the transmission of *Plasmodium*, the malaria-causing pathogen in these mosquitoes. Recently, Adolfi et al. (2020) have increased the effectiveness of a CRISPR-based gene drive system in female mosquito progeny resulting in mosquito populations resistant to transmitting malaria parasites.

(c) *Engineered gene drive for an agricultural pest.*

Buchman et al. (2018) developed an engineered gene drive system in *Drosophila suzukii*, a major worldwide crop pest. The gene drive system could maintain itself at high frequencies in a wild population and is being tested under contained conditions.

(d) *Genetically engineered bio-containment systems in mosquitoes.*

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. After release into the field, progeny die in the absence of the dietary additive (Alphey et al., 2010). 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. This may be essential where adult females are damaging, may also provide some efficiency improvements (Rendón et al., 2004), 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).

(e) *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 gene editing approach.

(f) *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

[12] http://www.targetmalaria.org

[13] https://ucimi.org

architecture, increased flowering and a lodging-tolerant stature amenable for responding to more inputs to improve yield (Stanic et al., 2020).

3.2.3. Research.

(a) *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 consortia[14] investigates and engineers interactions between nitrogen-fixing bacteria and plants. Amongst many developments, 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. Eseverri et al. (2020) have used synthetically designed genes to achieve and optimise the production of active nitrogenase protein in the chloroplasts of tobacco plants. 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 construction of synthetic beneficial microbiota for several crop plants (Waltz, 2017).

(b) *Insect delivery of modified viruses for the modification of crops (horizontal environmental genetic alteration agents [HEGAAs]) for biodefence and agriculture.*

The USA's Defence 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 June 2020. The authors used a modified *Tobacco rattle virus* encoding synthetic guide RNAs (gRNA) into *Nicotiana benthamiana* expressing the *Streptococcus pyogenes* CRISPR-associated Cas9 protein to demonstrate that heritable changes to mature plant chromosomes is possible (Ellison et al., 2020).

In similar research[15], *Potato virus X* 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. Progeny virions recovered from infected plants were able to cause changes in newly infected plants (Ariga et al., 2020; X. Ma et al., 2020). In addition to inducing genomic changes, epigenomic editing is now possible. A modified Tobacco rattle viral vector encoding gRNA was used to induce DNA demethylation in modified *Arabisopsis thaliana* cells; however, the modified epigenetic pattern was only heritable at low levels (Ghoshal et al., 2020).

(c) *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 seven active de-extinction projects globally (B. Novak, 2018): 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). Further, the same approaches are being applied to species close to extinction. In a recent report, a Przewalski's foal (*Equus przewalskii*), a species native to central Asia that went extinct in the wild and is still critically endangered with only about 2,000 remaining, was successfully cloned and born in San Diego Zoo, USA earlier in 2020[16]. The foal had

[14] https://synthsym.org

[15] The research was not funded by the DARPA Insect Allies Project.

[16] San Diego Zoo scientists revive cells from 40-year deep freeze to clone endangered horse, Los Angeles Times, 13 October 2020. https://www.latimes.com/california/story/2020-10-13/san-diego-zoo-scientists-use-cells-frozen-away-for-40-years-to-clone-endangered-przewalskis-horse.

been cloned from skin cells taken from a stallion in 1980 and safeguarded at the Frozen Zoo, the zoo's repository of 10,000 cell lines from more than 1,100 species and subspecies. Not all these projects involve synthetic biology techniques, but tools such as genome editing could accelerate the results. Although de-extinction has not yet been achieved beyond viruses, conservationists and synthetic biologists already began discussing the potential impacts on biodiversity and ecosystems (Friese & Marris, 2014) and guiding principles on creating proxies of extinct species for conservation benefit are now in place (IUCN SSC, 2016).

(d) *Transient modification of agricultural plants through RNAi spray or nanomaterials.*

Synthetic double-stranded RNA (dsRNA) molecules targeted to a specific plant pest can be applied as a foliar spray. The vascular system of the plant then naturally translocates the RNA molecules, triggering a transient pest-resistant property (Cagliari et al., 2016). This transient modification of plants using topical application of dsRNA has been proven in the model *Arabidopsis* (Dubrovina et al., 2019). Recent studies 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 potyvirus bean common mosaic virus (Worrall et al., 2019); insects, such as Colorado potato beetle (Petek et al., 2020) or soybean aphids (S. Yan et al., 2020), and; fungi, such as *Fusarium graminearum* (A. Koch et al., 2016) or *Botrytis cinerea* (M. Wang et al., 2016). Each example is an important first step towards developing practical applications of this approach in crop protection.

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 *Nicotiana 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 nanostructure are also being assessed as a biomolecule delivery method. Zhang et al. (2019) delivered siRNA and effectively silenced a constitutively expressed gene in *Nicotiana benthamiana* leaves using a DNA nanostructure.

(e) *Genetically engineered plants to produce recombinant polyclonal antibodies against snake venom toxins.*

Julve Parreño et al (2018) described an synthetic biology approach affordable and cost-effective antivenom production based on plant-made recombinant polyclonal antibodies. This approach has the potential to overcome the shortage of supply of antivenoms developed from the plasma of hyperimmunised 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.

(a) *Biopharma.*

- Semi-synthetic artemenisin (*Amyris*).

  Yeast metabolism was engineered to express the biosynthetic pathway to produce the anti-malarial compound artemenisin. Commercial production began in 2013 (Kung et al., 2018; Zeng et al., 2008).

- Human cells for therapeutic purposes, such as for CAR-T cell therapy for cancer (*Novartis*).

  T-cells are re-programmed 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).

- Synthetic tissues and organoids (*Cellbricks*).

3D printed biopolymer cell culture scaffolds for synthetic tissues and whole synthetic organoids for research or therapeutic purposes (Ebrahimkhani & Ebisuya, 2019; Kloke, 2019; Kloke et al., 2019).

- 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 to extracts from Horseshoe crab blood (limulus amebocyte lysate [LAL]; *Lonza*, *bioMérieux*).

  The Factor C sequence was cloned from horseshoe crabs and the protein was engineered to active upon binding 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).

- 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).

(b) *Carbon recycling.*

- Engineered algae as biofactories for renewable fuel (*Synthetic Genomics*, *Photanol*).

  By applying multi-omic approaches for strain characterisation and genomic manipulations (BioBricks, genome editing), algae can be metabolically engineered or contain genetic circuitry for optimised bio-fuel production. (Benders et al., 2016; Jagadevan et al., 2018; Savakis et al., 2013; Watts et al., 2007).

- 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, chemo-autotrophic, and photoautotrophic) are metabolically engineered to convert one carbon gases into chemical precursors (Dürre, 2017; Humphreys & Minton, 2018; Karlson et al., 2021; Kim et al., 2016; Oakley, 2012; Reed & Dyson, 2013). For example, cells can also be programmed to produce limonene, a jet fuel (Jansson et al., 2019).
- Greenhouse and waste gas (CO_2, CO, CH_3) capture and conversion into bacterial-based soil fertilisers (*Kiverdi Inc*).

  Metabolically engineered bacteria capture a variety of atmospheric and waste gases to synthesise fertilisers and bio-stimulants for plants and fungi. The products are excepted to increase soil carbon and nitrogen, among other nutrients (Molitor et al., 2019; Dyson et al., 2019).

(c) *Fabric.*

- Bacteria that turn methane into bioplastics for clothing (*Mango Materials*).

  Bacteria were metabolically engineered to consume methane for the bioproduction of plastic precursors, such as polyhydroxyalkanoate, for clothing (Hickey et al., 2018; Pieja et al., 2017; Zhang et al., 2020).

- Synthetic silk from microbial sources (*AMSilk*, *Spiber*, *Bolt Threads*).

  Recombinant spidroins (spider silk protein) and protein fiber yarns were engineered for bacterial production and enhanced qualities (strength, flexibility, etc.). Production occurs in microbial hosts, such as *E. coli* (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 re-programmed 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 tomelting temperature and elasticity (Wendy Craig et al., 2017; 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; R. 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 the engineered proteins were assembled from rare or extinct plants (Lecourt & Antoniotti, 2020; Ridley et al., 2019).

(e) *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). For 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 CO2 Aquafeed by *Kiverdi Inc*.).

  Metabolically engineered bacteria capture atmospheric CO_2 with the assistance of H_2-oxidising bacteria to produce amino acids and proteins. Renewable energy powers the hydrolysis of water to produce H_2 gas for the hydrogen-oxidising 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*).

  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)

(f) *Part, 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[17] and Biomaster database[18] (Wang et al., 2021) have comprehensive and updated catalogues of such parts.
- Expanded CRISPR-Cas systems for genome editing and diagnostics (*Mammoth Biosciences*).

  Novel CRSIPR proteins and systems are 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 for a test for SARS-CoV-2 developed in collaboration with GlaxoSmithKline (Broughton et al., 2020; East-Seletsky et al., 2016; Mammoth BioSciences, 2018).
- Olfactory detection devices (*Koniku*).

  Konicore™, 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. Advanced development.

(a) *Cyanobacteria production platforms in contained environmental facilities.*

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.

(b) *Bio-fabricated 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 utilising induced pluripotent stems re-programmed 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 (Lixin 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).

[17] http://parts.igem.org/

[18] http://www.biomaster-uestc.cn

(d) *Plant-based vaccines (Medicago).*

Virus-like particles (VLPs) are recombinantly produced viral structures that exhibit immunoprotective 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 are the recombinant virus-like particles for vaccine production against COVID-19 and influenza produced in transiently modified tobacco plants. Vaccines against seasonal influenza have been registered, but not approved in any jurisdiction, and vaccines against SARS-CoV-2 are in late stages of clinical trials (D'Aoust et al., 2016; Makarkov et al., 2019; Tusé et al., 2020)

(e) *Engineered phages as anti-microbials (Eligo Biosciences).*

Engineered phages have also been deployed to deliver CRISPR-Cas nucleases to act as sequence-specific antimicrobials (Bikard et al., 2014; Bikard & 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 be designed to excrete engineered proteins (Charbonneau et al., 2020; Claesen & Fischbach, 2015; Whitfill, 2019).

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; B. Y. 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 compartmentalisation and biocatalytic activity enables new strategies in bottom-up synthetic biology, regenerative medicine, pharmaceutical science, and biotechnology. Further, the Build-A-Cell Consortium[19] is an open community that supports the science and engineering of synthetic cells.

(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 be used in bar-coding and combinatorial tagging, retrievable information storage, and self-assembling nanostructures (Hoshika et al., 2019).

(c) *Re-creation of an extinct infectious horsepox virus from chemically synthesised 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 horsepox virus 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 microorganism (in this case, synthesising HPXV *de novo*) was a way to proceed with research without comprising regulations.

[19] https://www.buildacell.org/

(d) *Genetically engineered bio-containment 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). 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 USA 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 amongst iGEM teams to respond to biosafety concerns (Guan et al., 2013). Further, unnatural nucleotides can be used to prevent transfer transgenic information to wild-type organisms as the 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).

(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[20] (Church et al., 2012), 739 kilobytes of computer files[21] (Goldman et al., 2013)and 35 files[22] 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 3-D 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 (J. 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 synthesise DNA *in vitro* (Yim et al., 2021).

(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 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

[20] 53,426 words, 11 JPG images, and one JavaScript program

[21] 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).

[22] 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://www.nordgen.org/sgsv).

platforms in bioremediation and biorefinery, and Lin et al. (2020)reviewed portable detection biosensors with cell-free synthetic biosystems for detecting environmental pollutants. Interestingly, 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.

Considering that the applications listed in this section is not an exhaustive listing, a comparison between 2015 (date of publication of the Technical Series No. 82 on Synthetic Biology) and this present document indicate that the number of synthetic biology applications commercially available and in advanced development has greatly 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 amongst others. This marks a shift from the biofuels previously profiled. Although some biofuel products continue to be available, some companies specialising in algal biofuels (e.g. Solazyme and Calysta) have been sold or have gone out of business. Thus, the availability of these products is unclear. Developments continue with other microbes, but the focus may have shifted to bio-producing 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 bio-fabricated versions. Additionally, new devices designed for olfactory detection or 3D printing tissues 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, engineered bacteria fertilisers and self-limiting insects. Other products are in advanced stages of development, such as genome edited animals and organisms containing engineered gene drives to control vector-borne diseases. The latter of which was previously in early stages of research at the time of publication of the previous technical series.

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

D. POTENTIAL IMPACTS OF COMPONENTS, ORGANISMS AND PRODUCTS RESULTING FROM SYNTHETIC BIOLOGY

Parties of the CBD have recognised 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 on Biological Diversity (CBD). The text of the CBD (Article 2; United Nations, 1992) 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 recognised that the conservation of biological diversity occurs at all levels: genes, species, and ecosystems.

Furthermore, in the context of the CBD, 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; United Nations, 1992). Sustainable use encompasses ecological, economic, social, cultural, and political factors (Glowka et al., 1994).

The consideration of potential impacts from 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 which are equally important for decision-making and governance of the issue. This section will therefore cover both impacts on biodiversity and social, economic, and cultural (SEC) concerns from synthetic biology applications.

4. Applications of Synthetic Biology and Their Potential Impacts on the Conservation and Sustainable Use of Biological Diversity.

Although synthetic biology is often referred to as a coherent and single discipline, the numerous areas of synthetic biology research 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). 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 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 in advanced stages of development as well as on applications that are widely discussed at the international level (i.e. the use of engineered gene drives for conservation purposes 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 applications of synthetic biology on the conservation and sustainable use of biodiversity.

4.1. Species elimination, suppression or displacement.

Applications containing engineered gene drives are being designed to modify, suppress or eradicate populations of various target species (Rode et al., 2019; Scott et al., 2018). The development of applications based on engineered gene drives targeting invasive alien species (IAS), human disease vectors and agricultural pests are currently under development (see Section 3). Potential target species include invasive rodents (e.g., invasive house mouse or black rats that threaten biodiversity on islands; Leitschuh et al., 2018), 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). Worthy of mention is that 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 commercialised. However, these developments respond in part to the fact that the toll of loss of biodiversity (especially on islands), human suffering, and lowered crop productivity from some of these organisms is extensive (Friedman et al., 2020) as well as the fact that some envisioned environmental applications (e.g. those from newly developed gene-editing

techniques such as CRISPR) may bring benefits for ecosystems (Kofler et al., 2018), and could also be a tool to control threats to biodiversity.

In the specific case of IAS, recognising that they are a leading cause of biodiversity loss, arresting their adverse effects is a priority for governments worldwide, and programmes aimed at controlling or eradicating IAS therefore continue to be deployed across vast areas of the globe. As a consequence, the development of effective methods to suppress, displace or eradicate populations of IAS has been a key conservation goal for decades, especially if approaches can be developed which produce less unanticipated and undesirable results than current control measures by conventional means (Reynolds, 2021).

In addition to the management of established IAS that are currently impacting biodiversity, synthetic biology also offers novel potential approaches for the rapid response and eradication of new IAS incursions, as synthetic biology applications may offer more tactical and targeted approaches and at 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).

However, it should be noted that the degree of impact resulting from synthetic biology applications to the management of IAS will likely 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 species is native or performs an essential role in community structure and/or ecosystem dynamics (Redford et al., 2019)). Further, depending on the type and scale of the modification, gene-edited organisms released into the environment for instance, could also result in unwanted impacts on biodiversity, including off-target mutations, evolutionary resistance, ecological disturbance and extinctions; each of which have triggered a heated discussion regarding their environmental impacts and regulatory oversight (Esvelt et al., 2014; Kofler et al., 2018; Romeis et al., 2020).

Engineered gene drive systems, notably CRISPR–Cas9 gene drives, have recently emerged with potential applications not only in conservation but also in public health and agriculture (López Del Amo et al., 2020). This approach may increase the feasibility of large-scale control with genuine potential for continental-scale eradication of unwanted wild populations or species (Esvelt et al., 2014; Reynolds, 2021b). Engineered gene drives are also particularly appealing for release on islands which are the primary sites of vertebrate extinctions and where the limits of current control technologies are being reached but can ensure that the impact is better contained geographically (Esvelt & Gemmell, 2017; Godwin et al., 2019). However their use elsewhere raises concerns in terms of potential spread (Critical Scientists Switzerland et al., 2019; Dolezel et al., 2020).

Organisms containing CRISPR-engineered gene drives have not been released into the wild, but their development has stoked fears that even well-meaning applications of the technology, such as attempts to reduce populations of organisms that spread disease, could have unintended consequences for ecosystems (Callaway, 2018). The most advanced application is 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, ensuring that the modification is spread throughout the specific target populations relatively quickly and is effectively 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 (World Health Organization, 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 hybridisation to sibling species) need to be further explored in order to assess the degree of potential negative impacts (Roberts et al., 2017). For instance, 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. Thus, although many predators of mosquito larvae and adults may be

polyphagous i.e. they consume a variety of prey, there are species that specialise 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 their prey. Whether many more such highly specialised predators exist remains an open question. Similar mosquito dependency by other such specialised predators requires further investigation (Critical Scientists Switzerland et al., 2019) . 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, in attempting to understand the extent of impact on biodiversity, it is important to differentiate between the types of engineered gene drive system being utilised, as the application could theoretically either spread through target populations (non-localised) and persist indefinitely (self-sustaining) or be restricted in spread (localised) 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 (Devos et al., 2020; EFSA GMO Panel, 2020; P 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 population. They may have the same ultimate goal, e.g. eradication of an invasive species or pest, but they have different implications for potential environmental interactions. For example, a CRISPR-based eradication drive may spread into a non-target population or species (Noble et al., 2018; Rode et al., 2019, 2020)whilst a modification drive may alter the target population in an unexpected, detrimental manner (Gurwitz, 2014).

The survival in the wild of a synthetic biology organism containing an engineered gene drive will depend on, amongst others, how well it can compete with its wild relatives and its susceptibility to mutations that lead to the loss of either the desired trait or that disrupts the synthetic change. Limiting the scale of exposure of the target organism in the environment, either by the number, timing and location of releases may minimise the extent of impact (Brandenberg et al., 2011).

However, recent research indicates that engineered gene drives may face resistance and limited efficacy in wild mosquito populations. Resistance towards engineered gene drives is important especially for homing endonuclease and RNA-based methods. Both are extremely sensitive to mutations or genetic variability in their recognition sites; this will likely affect the spread of drives based on such mechanisms, at least for simple designs. These phenomena have already been observed in the first laboratory experiments. Further, CRISPR-Cas9 engineered gene drives – and homing endonuclease genes in general - are prone to the development of resistance alleles that are immune to conversion by the drive system (Hammond et al., 2017). Moreover, engineered gene drives that bring a fitness cost are expected to accelerate resistance development. Thus, in the case for suppression drives, it is expected that there would be great evolutionary pressure. So, the concern that potent engineered gene drives, once released, would potentially act globally should therefore be nuanced and is case-specific; they might spread through different populations but not necessarily achieving very high frequencies in each (Rüdelsheim & Smets, 2018). However, it is rather uncertain how rapidly modification drives would spread into the wild owing lessened selective/evolutionary pressure (Critical Scientists Switzerland et al., 2019).

Extending the discussions to broader impacts in the ecosystem, 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, biological control, decomposition) (Romeis et al., 2020). Further, the reduction of the target organism populations may result in the increase of populations of other species (niche

replacement). Removing one vector/pest species could allow another potentially harmful species to take its place. In this respect, technologies for population replacement instead of population suppression are likely to induce less ecological harm as the target species is still present albeit in lower numbers, therefore no empty niche is created (Kopf et al., 2017; Rüdelsheim & Smets, 2018; EFSA GMO Panel, 2020). Moreover, the reduction of abundance (or extinction) of the target species can have consequences for e.g. 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 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).

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. Some gene drive-engineered organisms might persist for only a limited number of generations. Others 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). Concerns over the lack of controllability have been raised by gene drive developers who have reported that gene drives are likely to be 'highly invasive' and spread to most interbreeding populations (Noble et al., 2018; Sirinathsinghji, 2019). It has also been noted that if drives do propagate as intended, there is considerable uncertainty about the extent of the geographic areas they will eventually inhabit and the numbers of species that might be affected(Critical Scientists Switzerland et al., 2019). Given that organisms containing engineered gene drives can potentially impact biodiversity, national sovereignty and food security, there is a crucial need to develop strategies to minimise any potential risk, including those of intentional and unintentional spread and to mitigate harm to humans or the environment (de Wit, 2019; DiCarlo et al., 2015; National Academies of Sciences Engineering and Medicine, 2016).

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 fertiliser, Oxitec's genetic biocontrol insects), to those that increase nitrogen fixation efficiency in crop symbiotic bacteria (Ryu et al., 2020), that improve yields of current cropping areas through modified crop architecture (Stanic et al., 2020), and that provide alternative weed control (e.g. Cibus' oilseed rape resistant to CLEARFIELD® herbicides). Additional potential benefits include: enhancement of decomposition rates and nutrient fixation; reduction in the application of fertiliser; 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 observed from analogous applications exploiting genetic modification technology in agriculture. These 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 Environment Policy, 2016).

Some of the techniques of genome editing (that for some may not fall under synthetic biology; see Scope & Methods) are less precise than others such that additional molecular changes to the intended (i.e. off-target modifications) can also be introduced into the host organism; again, phenomena that have been reported with classical genetic engineering (Eckerstorfer, Heissenberger, et al., 2019). 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. Mutagenesis techniques[23] used in conventional breeding are rife with off-target effects that few people ever bother to detect or characterise. These historically have not been a cause for safety concerns, and there has been a history of safe use of mutagenised crops (Duensing et al., 2018). Thus, while it is possible to optimise the editing process to minimise 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 lead to phenotypic effects affecting the properties of the modified organism (European Commission High Level Group of Scientific Advisors, 2017), and have the potential to ultimately lead to alterations of population characteristics, especially when spread amongst individuals via gene transfer. This may ultimately lead to unintended or unexpected consequences during interactions with associated species or populations in the surrounding environment. Conversely, the cause of some of this imprecision is also being 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). Additionally, in the case of *de novo* domestication or domestication of new crops, it has been suggested to further investigate the presence of any potentially toxic secondary metabolites from the wild relative and whether the food product is digestible in the final breeding line (Fernie & Yan, 2019; Van Tassel et al., 2020). Further, there have been concerns raised that both unintended on-target and even precise edits could potentially affect cellular regulation, which in turn could affect the protein composition and thus safety of the plant in the environment (African Centre for Biodiversity, 2020; Canadian Biotechnology Action Network, 2020).

4.3. Climate change and environmental solutions.

As is the case for other environmental challenges, synthetic biology has the potential to help tackle 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 time scale 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 time scale (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 (K. R. N. Anthony et al., 2020; Matz et al., 2018). As described earlier (Section 3.1.3(c).), 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 the absorption of elevated atmospheric

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

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 maximised (Oppen et al., 2017). On the other hand, since it is not feasible to engineer millions of species, prioritising 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 genetically modified 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 (Secretariat of the Convention on Biological Diversity, 2020a). 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; which are 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 (Sections 3.3.1(b). & 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), which could then replace petrochemical sources or mitigate emissions caused by their combustion (Köpke & Simpson, 2020).

Potential negative impacts could result from the increased utilisation 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 considerable 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 escape 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 minimised as production conditions would be more strictly controlled (Shuba & Kifle, 2018). However, it is important to consider that these operations could be energy intensive and produce higher lifecycle 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 reduced between 60 and 90 % of conventional fossil gasoline, depending on whether the source was industrial off-gas or fermented biomass (switchgrass, corn 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 (AirProtein and CO2 Aquafeed) 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 (fertilisers, pesticides and herbicides) and arable land. Land use dependency could also be further minimised 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 supply 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, 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 synthesised item be a good substitute for the wild product, this could be positive for conservation, taking pressure off the wild species while supplying market demand.

However, the situation is a little more nuanced than originally expected. For example, the replacement of natural products with products resulting from synthetic biology could lessen the pressure on natural habitats but could also disrupt *in situ* conservation projects. Further, it was recently flagged within the CITES community that there may be the 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 (Section 3.3.1(d)) arose 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 inter-cropping 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) seems to support 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 Section 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 socio-economic aspects. A science-based assessment of impacts is therefore seen as part of a wider decision-making activity; one that evaluates such economic, political, moral and ethical concerns alongside scientific predictions of changes that would result

from using technology. Guidance on the process for assessing such concerns has recently emerged (Secretariat of the Convention on Biological Diversity, 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 socio-economic 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 promises to address global challenges related to the environment, conservation, climate change, health and more, international discussions concerning synthetic biology have come to the forefront and are now much more visible and have drawn attention from a wide range of actors. Their interests and concerns have a role to play in regulatory decision-making concerning activities involving synthetic biology products, the inputs for which extend beyond those addressed during a risk assessment (Section 6.). Typically, such decisions would be taken by risk managers or decision-makers, given the characterised 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). A recent example is the ongoing conversation about the responsible application of CRISPR that is taking place at both national and international levels concerning the limitations of current global governance structures to safeguard its use. Largely missing from this conversation however, is attention to local communities in decision-making which are likely to be the first to feel any potential impact from these applications (Kofler et al., 2018). 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 (Section 5.1.2.). Conversely, the synthetic biology community needs to 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).

As suggested by Oye *et al*. (2014), for emerging technologies that affect the global commons, concepts and applications should be published in advance of construction, testing, and release. 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 adaptation of regulations and conventions in light of emerging information on benefits, risks, 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 (Secretariat of the Convention on Biological Diversity, 2015); Secretariat of the Convention on Biological Diversity, 2018). This same approach continues to be echoed, for example in a recent survey of experts (Lassoued et al., 2019) in which the majority indicated that the regulations for health and safety, followed by export markets, consumers, and the media play a major role in determining where and how New Breeding Techniques[24] (Obukosia et al., 2020; Seyran & Craig, 2018), including genome editing, will be developed and used in agriculture. 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 (Section 6.), that then dictates the characteristics of new products or technologies from synthetic biology to be assessed both scientifically (Craig et al., 2017) and socio-economically (Secretariat of the Convention on Biological Diversity, 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 authorisation is considered (Office of the Gene Technology Regulator, 2013). This would be extremely helpful, especially in cases where policy objectives are in

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

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 displacement 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 people), 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 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[25], 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 recognise the plights of Indigenous peoples and defend their rights (George et al., 2019), initially by the Indigenous and Tribal Populations Convention no. 107 of the International Labour Organization adopted in 1957[26] as revised in 1989 by the Indigenous and Tribal Peoples Convention no. 169, and subsequently by the Convention on Biological Diversity in 1992 and the United Nations Declaration on the Rights of Indigenous Peoples adopted by the UN General Assembly adopted September 2007 (see Sections 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 organisations. 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 it notes that elements within FPIC are interlinked and should not be treated as separate elements (FAO, 2016). Similarly, after a 10- to 12-year process under Article 8(j) (see Section 8.1.7), the CBD developed 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" and "approval and involvement" for obtaining a fair and equitable share 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); independently decided upon (without pressure or manipulation; free), and; 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 IPLCs are involved through their full and effective participation in discussions and decision-making (FAO, 2016; SCBD, 2019a). Additionally, "prior and informed consent" was also enshrined in the Akwé: Kon Voluntary Guidelines, which were developed under the CBD 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 IPLCs (Secretariat of the Convention on Biological Diversity, 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 however 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

[25] 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.

[26] International Labour Organization, Indigenous and Tribal Populations Convention (No. 107) https://www.ilo.org/dyn/normlex/en/f?p=NORMLEXPUB:12100:0::NO::P12100_ILO_CODE:C107

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). Additionally, the 2018 COP 10-14 decision acknowledged the need for FPIC of IPLCs in relation to the release of organisms containing engineered gene drives (see Section 8.1.7.). Yet significant challenges remain in operationalising participatory decision-making and the FPIC of IPLCs.

Recognising 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 assessment 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 USA's 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, 2016). 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 publics, 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 engineered gene-drive modified organisms, 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 IPLC and participatory decision-making, however gene drive researchers and developers have begun to pursue strategies of engagement that resemble some of the principles of FPIC (George et al., 2019). For example, Target Malaria has organised and funded a strategy of engagement 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[27]. Similarly, The Mice Against Ticks project[28] has sought community consent prior to the development of a genetically engineered mouse to interrupt Lyme disease transmission, and community steering committees have been formed to guide the research. The need for more robust and standardised approaches to participatory decision-making in the context of synthetic biology applications is considered further in Section 11.

[27] https://targetmalaria.org/what-we-do/our-approach/#stakeholder-engagement

[28] https://www.media.mit.edu/projects/preventing-tick-borne-disease-by-permanently-immunizing-mice/overview/

5.2. Economic concerns.

5.2.1. International trade.

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity contributes to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms (LMOs) resulting from modern biotechnology. Of note, the Protocol establishes core procedures and a set of standards relating to the import and export 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), and the Agreement on the Application of Sanitary and Phytosanitary Standards (SPS) are 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 (for further information on these Agreements, see Section 9.). The different fundamental objectives of the international trade and environmental regimes have led to conflicts 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 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/policy-making options on the economy (considering trade, agro-industrial innovation and productivity) (Whelan & Lema, 2017). A formal analysis of the trajectory or dynamics that the interpretative flexibility is taking may be useful to anticipate the social perception of these decisions (Duensing et al., 2018).

5.2.2. Production of analogues of naturally occurring molecules.

As was reported in Section 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 wild caught species (i.e. 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 (Wellhausen & Mukunda, 2009). The main economic drivers for this appear to be (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 displacement of some of the natural products (i.e. naturally occurring molecules obtained from plants) can potentially ease negative pressures on wild or cultivated species, but it can also displace cultivation practices, often in topical and sub-tropical 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 displacement 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 displacement 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 via fermentation in yeast (see section 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 if the synthetic product is not appealing to consumers as a perfect substitute for the wild-sourced product e.g. 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 'real thing' or it could lead to greater public acceptance of natural products, such as horns (Broad & Burgess, 2016; CITES, 2018).

In the specific case for synthetic horn products, Chen and Sas-Rolfes (2021) considered a theoretical, economic model for synthetic wildlife 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. They concluded that, overall, the reduced price of the products would decrease poaching. In addition, bio-fabricated 3D-printed horn could also be used by as a direct replacement for artisans, which could reduce poaching demand (Pandika, 2017). However, producers of synthetic horn products may prefer to keep prices at a level high enough that 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.

And finally, another example is the anti-malarial 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 that 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 (United Nations Conference on Trade and Development, 2019) of whether SSA will eventually eliminate, or significantly reduce, the market for the natural artemisinin product concluded however 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.

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.* 2009). 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; "Synthetic Biology: The Technoscience and Its Societal Consequences," 2009). 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

synthesised elements are able to out-compete natural ecosystems, "delivering more services with less biodiversity". 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 neighbouring country restricting its use is feasible and raises questions about governance and ethical issues that could be also related with the FPIC (see Section 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 utilisation 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 synthesise 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 synthesise DNA sequences and downstream metabolic intermediates and pathways also raises contrasting views regarding equitable access and benefit sharing associated with the utilisation 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 Section 8.1.5. below).

Ethical considerations of biodiversity and of how people relate to biodiversity are also recognised as important in the context of the CBD. For example, CBD COP10 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 IPLCs; the fair and equitable sharing of benefits with IPLCs; and the precautionary approach, including relevant IPLCs and the use of local criteria and indicators in the prediction and assessment of potential harms to biodiversity (decision X/42, Annex A, 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 on 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 around the possibilities to either using this technology irresponsibly and cause harm, or not using it at all, which could also prove damaging to humans, our welfare, and our planet (Kofler et al., 2018).

With regards 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 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 self-determination and being a moral agent. Additional concerns could also be related to the perpetuated 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 postnatal death, or perpetuate poor animal management in intensive farming operations (Cotter & Perls, 2019; Graeff et al., 2019). Further, 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 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. A recent IUCN report makes a plea for the policy debate to be grounded in evidence, emphasising 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*" (Redford et al., 2019). 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 (or dual-) use of synthetic biology. Bioterrorists might, for example, create 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 virus can be modified to evolve into a dangerous human virus (Herfst et al., 2012). It has even been suggested that pathogens might be engineered to attack only a particular genetic subset of a population (Garfinkel et al., 2007). Likewise, Mukunda *et al.* (2009) predicted that biological weapons customised to attack specific groups were highly likely to be developed in the long term (10 or more years), i.e. the period between the previous technical series document and this update. 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 gene editing technologies to subject market GMOs 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.

One such example is the "Insect Allies" program funded by DARPA, which utilises HEGAAs (see Section 3.2.3(b)). The aims of the project are to develop counter measures 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, 2021). 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 (BWC; Section 9.3(c)), 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 theorised that such applications could have food security implications if deployed (Reeves et al. 2018).

Beyond actions potentially coordinated by governments or organised groups, there are additional concerns over the potential emergence of a 'bio-hacker' 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, 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.

As of 2019, no country regulates the sales of synthetic DNA (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)[29], which implements a Harmonised Screening Protocol to voluntarily screen all orders in alignment with guidance[30] from the USA's 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 sub-sequences to a relatively short list 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. DNA obfuscation, based on cyber-hacking malicious code obfuscation[31], could allow an unknowing biologist to use a malicious sequence, leading to their exposure to a dangerous agent. In a proof-of-concept experiment, researchers were able to successfully order a toxic peptide from a DNA synthesis company (Puzis et al., 2020).

One 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

[29] https://genesynthesisconsortium.org

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

[31] Obfuscation is when code is purposely complicated to conceal what the it performs

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 "bio-hacker" garage, to a functioning product that can be disseminated widely. 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 (International Risk Governance Council, 2009; Secretariat of the Convention on Biological Diversity, 2015; USA National Academies of Sciences & Engineering, 2016; InterAcademy Partnership, 2018):

I. 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.
II. Dissemination may extend beyond academic and professional circles as biological engineering becomes more accessible. This may include less responsible individuals and organisations. 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-glamorisation 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 USA's Presidential Commission for the Study of Bioethical Issues (PCSBI) claims it is "easy to anticipate potential benefits" of synthetic biology to biosecurity, such as identifying biological agents of concern and countering biosecurity threats (PCSBI 2010). 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, accelerate vaccine production, and provide therapies for some pathogens (Endy, 2005; Mukunda et al., 2009). The latter 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 (Zhang et al., 2020).

6. General Biosafety Concerns Associated with Synthetic Biology Applications.

While Section 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, intended use and receiving environment. The suitability of existing risk assessment methodologies as well as potential management strategies are also discussed. The section does not intend to be a comprehensive list or guide 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 less 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. Therefore, the adequacy of current methodologies for the environmental risk assessment of synthetic biology products might depend on how their novelty and complexity is perceived (Wikmark et al., 2016). Different methods and techniques of synthetic biology may need different forms and levels of oversight. Thus, any new risk assessments, cost-benefit analyses and regulations must flexibly encompass different applications, uses and products (ETC Group, 2012). 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). These applications are challenging existing regulatory systems in an unprecedented fashion due to the need to address novel risks and impacts, the high 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). 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). 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).

The overall aim of a risk assessment is to identify, characterise 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, whilst the identified risk is characterised by considering how serious the harm could be (consequences) and how likely that harm could occur within the context of the case (A. 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, and the need for any measures to reduce it considered where pertinent. Risks are characterised by testing specific hypotheses on the probability that harm will occur and the severity of the harm if it occurs. 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 (Craig et al., 2017; Devos et al., 2019).

Although the risk assessment methodologies may differ between countries and their regulatory authorities, the risk assessment is a process based in science 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 living modified organisms as per the Cartagena Protocol, the 'Points to Consider' in Annex III of the Protocol (Secretariat of the Convention on Biological Diversity, 2000a; Section 8.2.) is a good summary of the types of information that are regularly considered during a risk assessment, and that may be extended/adapted to some applications resulting from synthetic biology.

Here below some considerations regarding risk assessment for three synthetic biology supporting technologies that have received considerable regulatory attention to date are presented.

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 invasive alien species 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 engineered gene drive organisms 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 organisms 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, 2016; 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, and thus the step-wise approach to environmental releases, as practiced with other types of LMOs, may require adaptation (Keiper & Atanassova, 2020). This opens unprecedented 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).

It has been reported that engineered gene drives designed to "*suppress or enhance a species population at a rate that is faster than natural ecological processes or evolutionary rates*" may require the definition of additional pathways to risk assessment endpoints (National Academies of Sciences Engineering and Medicine, 2017). Recently, the European Food Safety Authority (EFSA) noted that the temporal and spatial scope of gene drive-engineered insects once released precludes testing by observation at such scales. As such, they are concerned that the molecular characterisation, 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). These sentiments, and others, were echoed by the AHTEG on Risk Assessment under the Cartagena Protocol, who recommended the development of further guidance on applications featuring engineered gene drive systems (Secretariat of the Convention on Biological Diversity, 2020c). Although it recognised that existing risk assessment methodologies may still be applicable for such organisms, the group indicated that specific technical or methodological challenges require further attention, including: 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 recognised 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 of organisms containing engineered gene drives and its interaction with the environment, questions have been raised concerning whether risk assessment could result in sufficiently reliable conclusions (Dolezol et al., 2020). Further, the risk assessment of engineered gene drive organisms will also require the development of new tools to complement established methodologies, including the use of models to help predict the ecological consequences of released engineered gene drive organisms. Unlike non-engineered gene drive organisms which can be limited in time and space and therefore provide data from small-scale tests that can be relevant to large-scale releases, the potential of engineered gene drive organisms to spread over large areas and landscapes, 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 engineered gene drive organisms near potential release, further ecological work will be essential to enhance model predictions (Sánchez et al., 2020).

6.1.2. Genome editing.

Discussions on how to assess and regulate genome edited plants essentially revolve around two approaches. Those who consider (certain types of) genome edited plants of low or negligible risks and those who 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; Kawall et al., 2020)i.

When it comes to risk assessment considerations associated with potential unintended effects or off-target cutting 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). 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).

It's been argued that the current approach to risk assessment is not designed to detect unintended consequences of employing some new breading techniques (i.e genome editing ; Christ et al., 2018). In response to this, there have been proposals that untargeted metabolomics could be part of a routine protocol assessing gene edited crops. However, the use of untargeted metabolites in the characterization of these crops has also been subject of criticism (Court of Justice of the European Union, 2018; Lassoued et al., 2019; Marchant, 2001). In a separate proposal, risk assessments could be tailored to the levels of uncertainty to be expected. For example, a "risk assessment light" could be implemented for cases with minimal changes and familiarity with the particular trait or plant of use (Eckerstorfer, Dolezel, et al., 2019; Schiemann et al., 2020).Several traits beginning to appear in plants being developed from new genetic modification techniques (e.g. herbicide resistance, modified composition) are becoming particularly relevant, especially as some traits are already familiar from existing LM crops. Other traits being developed in plants however are novel; meaning they are not present in agricultural plants currently cultivated, and their underlying physiological mechanisms are not yet sufficiently elucidated. 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, Dolezel, et al., 2019).

As discussions about the safety of genome edited organisms continues 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. The USA National Academy of Sciences (2017) indicated that for products such as "next generation" GM crops, it was not anticipated that risk assessment endpoints would be different from previously assessed GM crops. In France, the Scientific Committee of the High Council for biotechnology, identified the following three points to consider in terms of hazards 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 similar risk assessment methodologies 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). But, in cases of SDN-1, SDN-2 and ODM, the changes could be equivalent to changes expected from classical breeding, thus may not pose unique challenges (Jones, 2015; D. Zhang et al., 2020a). However, a lack of scientific data on engineered animals, how animal systems respond to genome editing, mosaicism produced from animal cloning methods (e.g. somatic nuclear transfer) 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.

Debate over RNAi-based GM plants provides an example of the different points of view that emerged when discussing the approaches to assess potential impacts from this technology. While some regulators considered RNAi-based GM plants to be no different from any other GM plant (Heinemann et al., 2013), others have acknowledged that they might affect their present approach for risk assessment (EFSA, 2014). 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 commercialised GM crops. Risks associated with the intended changes in current GM crops are usually related to the newly expressed proteins (e.g., possible toxicity and allergenicity), however these are not necessarily relevant for RNAi-based plants (Arpaia et al., 2020; Casacuberta et al., 2015). Notably, 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).

EFSA organised an international workshop in 2014 to discuss potential risks associated with RNAi-based GM plants and to identify issues unique to their risk assessment. In their report, scientists and regulators highlighted that baseline data are key to inform the risk assessment of RNAi-based GM plants and that the knowledge on RNAi mechanisms is rapidly evolving but still lack sufficient knowledge of mechanisms governing mRNA-small RNA interactions (Casacuberta et al., 2015). In the meantime, Brazil, New Zealand, and Australia have approved RNAi-based GM plant events for environmental and food/feed commercialisation 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 used instead in a spray, it was noted that dsRNA is a natural biological molecule that is readily degraded in nature and biological systems, specific formulations to ensure its stability and effective delivery to targets will be required on a case-by-case basis (Taning et al., 2020). Thus, it represents a novel type of biological protection/"biopesticide" and it is important that safety assessments for plant protection products are 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 potential 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 are 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 datasets 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; J. 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. Due to the sequence specific nature of the technology, it is likely that resistance attributable to changes in sequence can be mitigated through a re-designed 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); and
- an understanding of epigenetic changes induced by exogenous RNAi (Dalakouras & Papadopoulou, 2020).
- 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.

Amongst synthetic biologists and in policy discussions, 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" (Secretariat of the Convention on Biological Diversity, 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 (Secretariat of the Convention on Biological Diversity, 2015). Some of these, and others, are discussed in following.

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. *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 lethality and synthetic resistance.

When considering engineered induced lethality (also referred to as "kill switch" or "suicide gene") (Section Section 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 and thus has implications for the design of genetically engineered bacterial product for environmental applications (Section 6.2.3.). The selective pressure acting to inactivate or lose suicide genes (i.e. 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". Approaches to use built-in biocontainment strategies in engineered gene drives are discussed next (Section 6.2.3.).

6.2.3. Genetic biocontainment approaches.

The co-incorporation of a genetically engineered bio-containment system offers an increased ability to help control the spread of engineered gene drives and mitigate derived risks. In fact, several risk mitigation strategies have been proposed with the most advanced applications being developed based on RIDL (Thomas et al., 2000) (Section 3.2.2(d).). 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 at 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 sensu [Esvelt et al., 2014]; overwriting drives [DiCarlo et al., 2015]) or both the drive and wild-type alleles (immunising 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 neutralising an engineered gene drive (Wu et al., 2016; 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 (Xu et al. 2020). In progeny assays, CATCHA converted *cas9* between 57 to 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 the authors suggested further design considerations are warranted due to unexpected and off-target effects (e.g. the use of two gRNAs, optimising gRNAs). For CATCHA, the authors hypothesised that some progeny where the CATCHA did not copy, likely contained non-functional *cas9* due to indels caused by non-homologous end joining (Wu et al. 2016). In the case of e-CHACR, a biased inheritance of donor chromosomes was observed and attributed to cutting twice or induced male or homozygous lethality (when located on the X chromosome). For ERACR, the action of the element damaged the chromosome, 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 damaged caused to the chromosome carried higher fitness costs than retaining the drive element (Xu et al., 2020). Thus, the same authors concurred with the recommendations of the USA National Academies of Sciences & Engineering (2016) that the decision to proceed with potential releases of engineered gene drive systems should not be predicated on constructing neutralising elements and that such systems should be developed only for precautionary purposes.

6.2.4. Post-release removal of synthetic biology applications.

Some synthetic biology developers are beginning to explore the possibility of developing mechanisms by which organisms 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), or underdominance drives (Heffel & Finnigan, 2019). In the case of the anti-drives, a standard reversal system only targets engineered gene drive individuals; immunising 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 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 engineered gene drives organisms within native ecosystems (Heffel & Finnigan, 2019).

6.2.5. Detection and identification of synthetic biology organisms.

Current 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, such as genome editing, may however lack integrations of any foreign DNA or corresponding genetic elements commonly used in classical genetic engineering. This has important repercussions for the effective detection and identification of synthetic organisms, and especially for those authorised for international trade (currently plant-based commodities). As explained earlier, the application of genome editing can introduce small changes and aims to minimise 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 (Y. 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).

If a known insertion is present, PCR-based methods will likely be the method of choice as they are highly specific and sensitive. Based on the experience from GMO testing, it should be feasible to establish event-specific PCR methods targeting larger nucleotide sequence changes induced by genome editing (for example SDN3). Short sequence changes (substitutions or indels of one or a few nucleotides) induced by SDN1, SDN2, 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). 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 concern, recent developments have demonstrated the potential possibility of detecting and quantifying genome edited canola and rice utilising real-time quantitative PCR and droplet digital PCR, respectively (Chhalliyil et al., 2020; Peng et al., 2020). In particular, the method for detecting the genome edited oilseed rape demonstrated consistency with ISO17025[32] standards (Chhalliyil et al., 2020).

Should a specific sequence that is different to the reference be detected, it may need to be clarified whether this sequence occurred naturally or whether it was introduced by a genome modification technique. As conventional mutagenesis techniques, such as irradiation or mutagenic chemicals, as well as genome editing applications, do not leave specific imprints in the genome, it may be 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. 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 (Ribarits et al., 2021). Others, however, consider the technological problems to be surmountable should there be the political will to do so (Kawall et al., 2020).

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

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-conceptualisation 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 (Section 3.2.1(d).), for visual detection via a suitable epi-fluorescent 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 lacking, technologies continue to be developed and could be tested for feasibility (Keiper & Atanassova, 2020).

E. SYNTHETIC BIOLOGY GOVERNANCE AND REGULATORY PERSPECTIVES

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 classical 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 classical 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 authorisation will be given. This section discusses the various regulatory approaches that are beginning to emerge following the commercialisation of the first applications of synthetic biology, along with challenges that have arisen as countries begin to decide how to best to utilise (or not) the various synthetic biology technologies.

7.1. Current regulatory practices and approaches related to synthetic biology.

As mentioned on 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 ethical, socio-cultural, epidemiological, ecological and economic considerations. Furthermore, potential risks of synthetic biology must be weighed against the benefits and considering that there could also be ethical components to the decision to use or not a new technology.

As regulatory authorisation 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 regulatory attention to date are genome editing, engineered gene drives and RNAi technology, and are discussed below.

7.1.1. Genome-editing.

A wide range of positions are taken by regulatory authorities in countries across the globe when addressing whether applications using genome editing will fall within their regulatory purview; positions that largely

depend in most cases on whether modifications appeared as natural mutations, untargeted due to radiation-based or chemical mutagenesis or targeted by the use of transgenesis or genome editing technologies (Custers et al., 2019; Menz et al., 2020). Those genome editing applications that do not aim at the insertion of foreign genes, but at inducing site-specific mutations at single loci of a plant's own genetic material, are able to create organisms that could have theoretically come into existence naturally or through conventional breeding. Thus, although 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 most countries (Duensing et al., 2018). Therefore, at one end of the range 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). Those countries have implemented such exclusions based on their implementation of the definition of "modern biotechnology" characterised by the Cartagena Protocol 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-GMO organisms (Keiper & Atanassova, 2020). For 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 2018, Brazil ruled that Corteva's 'waxy corn' developed through the use of genome editing was not an LMO, thus exempting the product from biosafety regulations (Comissão Técnica Nacional de Biossegurança, 2018). In the United States of America, a non-Party to the Cartagena Protocol, 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 USA Department of Agriculture announced that it has no plans to place additional regulations on genome-edited plants that could otherwise have been developed through traditional breeding prior to commercialisation. However, the USA Food and Drug Administration has proposed mandatory pre-market evaluations for all food animals whose genomes have been intentionally altered using modern molecular technologies, including gene 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 contain an animal drug, including those that acquire the alteration through cross breeding (FDA, 2021).

Similarly, in Argentina, Brazil, Chile, Colombia, Japan, Kenya, Russian Federation, South Africa and Israel (some of which non-Parties to the Cartagena Protocol) have established policies and/or guidance describing which genome-edited applications are not required to follow GMO regulation (in this case), and with especial reference to those where the final products do not contain foreign DNA sequences (Lema, 2019; Obukosia et al., 2020; Ku & Ha, 2020). 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 GM crops (Fritsche et al., 2018; Park et al., 2019).

Argentina updated their regulations on animal biotechnology in 2017 to include new technologies such as gene editing and so gene-edited animals will be subjected to risk assessment (Whelan et al., 2020). Running contrary to this, the Canadian regulatory process treats gene-edited animal products for food or feed uses no differently than their respective conventional animals or animal products under their regulatory process if no foreign DNA is present (Ellens et al., 2019). Likewise, Brazil's National Technical Biosafety Commission determined in 2018 that gene-edited hornless cows are conventional animals and that these cows and their products can enter the market (Genetic Literacy Project, 2020).

Developing countries, especially many in Africa, are gradually reviewing their regulatory frameworks in order to address genome editing and a preliminary appraisal indicates that many are most likely 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). 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 genetically modified organisms, they will have to still comply with relevant legislation to ensure their sustainability (Biosafety South Africa, 2019).

In China, discussions regarding regulation and risk assessment began in 2015. 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 GMO legislation. As of 2020 no formal regulations were issued regarding genome editing (D. Zhang et al., 2020b).

It has been argued that the detectability of genome edited products is technically more difficult compared to GMOs, and that therefore there is no point in having them regulated. However, recent advances in detection methodologies, including the adaptation of techniques already in use by laboratories, such as quantitative PCR and digital PCR, could facilitate the detection of genome-edited events more readily (Chhalliyil et al., 2020; Peng et al., 2020; Ribarits et al., 2020). In terms of policy-making, this argument is moot. Products are regulated because sectors of society want them to be regulated. If there are technical tools to detect the product, so much the better, but if not, regulation can also be based on a system of sworn statements, traceability, etc.

7.1.2. Engineered gene drives.

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

As these types of applications may spread across jurisdictional boundaries following authorised 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'etre*, posing different legal and regulatory challenges, because of their high potential to spread beyond national borders (Ching & Lin, 2019).

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

Also, while some groups are in favour of a moratorium of the environmental release of organisms containing engineered gene drives due to the apparent gaps in the regulatory oversight and the potential for serious ecological and societal effects (Civil Society Working Group on Gene Drives, 2016), others emphasise the potential benefits of gene drive applications and encourage further development and continued laboratory research (Dolezel et al., 2020).

So far, organisms containing engineered gene drives fall under the definition of LMO as per the Cartagena Protocol on Biosafety (Secretariat of the Convention on Biological Diversity, 2020c). Therefore, Parties to the Protocol need to regulate such organisms according to the provisions of the Cartagena Protocol. 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, which have begun substantive work specific to organisms containing engineered gene drives, are currently the best home for their international governance (Ching & Lin, 2019).

7.1.3. RNA-based technology.

GM RNAi plants are being assessed and regulated using existing regulatory frameworks. However, there is an urgent need to adapt existing plant protection products legislation so that it incorporates appropriate science-based risk assessment procedures for topical RNAi-based applications. 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 RNAi-based sprays, 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 regulation of RNAi 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 Malaysian Pesticides Act 1974. However, they also found that that plants or other organisms containing RNAi constructs would be considered transgenic in line with LMO legislation in both countries.

Thus far, two countries have taken specific decisions on exogenously applied RNAi sprays. 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 (J. A. Heinemann, 2019). In Australia, topically applied RNAi-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 authorisation of synthetic biology applications is expected to be made at the national level. It is important to remember however that other regulatory arena as well as other legal jurisdictions may have overlapping mandates (see Section 9.2.). For example, international law has an important bearing on the authorisation 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 fertilisation (for ameliorating climate change by promoting oceanic carbon dioxide uptake) under the Convention on Biological Diversity and the provisions of the Biological Weapons Convention (BWC). 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 for further discussions in this area.

An 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 harmonisation, 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 their 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

emphasises the need for responsible research and innovation, and proportionate and adaptive regulation for the maximisation of public benefit and minimisation of risk. It also suggests the development of technical standards at the national level to support the acceleration of commercialisation (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.

A series of reports from the USA's 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," the 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). The NASEM emphasised 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 synthetic biology does not raise new technical (or ethical) issues and was within the scope of the existing legislative scheme (Office of the Gene Technology Regulator, 2013). 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 timely responding to technological developments and ensuring regulation that is proportionate to risk (Gray et al., 2018).

The German Central Committee on Biological Safety in 2018 concluded that most synthetic biology approaches result in GMOs 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. Specifically, their assessment concluded that the insertion of synthetic genes, gene circuits, metabolic pathways, or entire genomes in an organism results in a GMO 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 bio-orthogonal systems are approaches that result in GMOs 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 GMOs 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 which provides a robust framework to regulate activities with synthetic organisms and their products. It was therefore concluded that discussions on synthetic biology are therefore considered in the context of biotechnology and in the legislative framework of biotechnology and GMOs (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 & moratoria.

In this section, illustrative examples of self-regulation by the scientific community commencing with the Asilomar Declaration in 1979 and subsequent calls for moratoria relevant to synthetic biology are provided. 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 amongst 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 the *Asilomar Declaration* (Berg et al., 1975) issued in 1975 at the Asilomar Conference on Recombinant DNA Molecules, and attended by 140 scientists predominantly from public institutions around the world, as well as lawyers, government officials and members of the media (Keiper and Atanassova, 2020). The moratorium involved deferring experiments on highly pathogenic organisms, toxic genes, and large-scale experiments, and containment safeguards for continuing research.

After Asilomar, precautions for 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 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 of uncertainties around hazards of rDNA, and the difficulty in obtaining accurate estimates of risk at Asilomar 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 "gentleman's agreements" in today's context of aggressive patenting, internet news, and global security conditions. Others lament that under 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 (Keiper and 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) agree to in 2000 at the fifth meeting of the Conference of the Parties (COP 5) to the CBD. They are also apparent in the moratorium agreed to halt major ocean fertilisation projects until scientists better understand the potential risks and benefits of manipulating the oceanic food chain, adopted in 2018 under the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (London Convention) and subsequently reinforced by COP decisions under the Convention addressing biodiversity and climate-related geo-engineering (Decision IX/16 in 2008 and X/33 in 2010).

Synthetic biologists have talked about self-regulation but have not made any concrete agreements. The 2006 "SB2.0" international conference on synthetic biology was initially anticipated to produce an "Asilomar-like" declaration, particularly with regards 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.

Discussions in recent years concerning self-regulation have focused on the environmental release of organisms containing engineered gene drives, with negotiators at COP 14 petitioned by over 200 mainly civil society organisations to consider contentious language that called upon signatories to "refrain from the release, including experimental release, of organisms containing engineered gene drives"[33]. 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 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*:

1) long-term studies to evaluate the effects of engineered gene drive use on genetic diversity in target populations, and

2) multidisciplinary teams of experts to develop scenarios on deliberate misuse environmental and security risks (K. A. Oye et al., 2014).

Conversely, other researchers caution the conflation of scientific assessment concerning biosafety with broader political and societal issues in favour of a more evidenced-based approach, and for discussions under the CBD 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 CBD. They call on stronger involvement by the scientific community in the CBD discussions as essential to support evidence-based decision-making and the development and/or adjustment of effective, adaptive and proportionate regulation (Keiper and Atanassova, 2020). While there is therefore no consensus amongst 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).

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 organisation with the objective of promoting the beneficial application of gene synthesis technology while safeguarding biosecurity. Representing approximately 80 % of commercial gene synthesis capacity world-wide, IGSC members apply a common protocol to screen both the sequences of synthetic gene orders and the customers who place them. It collaborates with national and international government organisations and other interested parties to curate a Regulated Pathogen Database derived from international pathogen and toxin sequence databases (International Gene Synthesis Consortium, 2017). Industry bodies such as the Biotechnology Industry Organization support commercial surveillance which are 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 minimise risk and maximise 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 a report on "Biodefence in the Age of Synthetic Biology", the USA's National Academies of Sciences, Engineering and Medicine (2018) observed that synthetic biology is being pursued overwhelmingly for beneficial purposes, ranging from

[33] 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

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. iGEM.

The world's largest international synthetic biology competition, known as iGEM (the international Genetically Engineered Machines competition) which has been running annually since 2003, attracts around 6000 students and community lab members form over 300 multidisciplinary teams from over 40 countries to compete in synthetic biology design, implementation, and integration into society using interchangeable biological parts and standard molecular biology techniques (iGEM, 2021).

As mentioned in Section 1.8., iGEM has implemented 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 recognise 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[34], 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 organisation 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, anti-microbial resistance and biosafety level 3 and 4 organisms)[35].

The 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 (e.g. "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)[36].

7.4. Intellectual property considerations related to 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). A decade or so later the storm does not appear to have materialised, however, 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

[34] https://2020.igem.org/Safety/Committee

[35] As per their Safety Rules: https://2020.igem.org/Safety/Rules

[36] https://igem.org/Safety

(Henkel & Maurer, 2007; Kenneth A. 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). There certainly appear to be valid concerns voiced by civil society and public sector organisations 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 in pro-poor products reaching the market 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 and Maurer 2007; Henkel and Maurer 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 are transforming life-sciences research (Sherkow, 2018), including synthetic biology applications which are approaching commercial release, for example, engineered gene drives (see Sections 1.4 and also 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 heavily relies on patent protection and is exemplified by the approach of the J. Craig Venter Institute (Gibson et al., 2008; Gibson et al., 2010; Glass et al., 2007). While working at the USA's National Institutes of Health in the 1980s, J. Craig Venter attracted attention and criticism for leading patent applications of thousands of short DNA sequences (Calvert, 2012). In 2007, scientists at his institute 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 creation of synthetic organisms for the production of biofuels like ethanol and hydrogen (van den Belt, 2013). The other main model is the BioBrick system, 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 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 standardised legal tool intended to facilitate sharing of bio-materials on an open basis by researchers, institutions and broader communities, by relaxing restrictions on redistribution and commercial use (Kahl et al., 2018).

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 organisations (continuing 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). A strong concern of civil society groups is that strong IP regimes could also restrict access to information for carrying out independent, effective risk

assessments (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 Section 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 on 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 fair and equitable sharing of the benefits arising out of the utilisation 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[37]. Depending on the scope of synthetic biology's definition (including the operational definition developed by the Ad Hoc Technical Expert Group on Synthetic Biology and acknowledged by the Conference of the Parties)[38] , the provisions of the Convention most relevant to the governance of synthetic biology are outlined below in Sections 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 approved by the Conference of the Parties at its fifth meeting in 2000, pursuant to 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 recognises 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 Section 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 minimising 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*

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

[38] As noted in the scope and methods in Section B.

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 Section 9.3.1(a)., "significant" under international customary law could be understood as a threshold exceeding *de minimis* threshold and that requires a certain intensity of impact. As has been discussed above, the probability of potential negative impacts of synthetic biology techniques is unclear for many applications. In addition, the interpretation 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.

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 negotiation, in response to Article 19, paragraph 3 of the Convention, and subsequent on-going implementation of the Cartagena Protocol on Biosafety (SCBD 2005). The Convention itself addresses biosafety through Articles 8(g) and 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 living modified organism 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 (Article 2). According to the IUCN *Guide to the Convention on Biological Diversity*, this definition was "designed to include both present and future technologies and processes" (Glowka et al., 1994). The Convention does not define "biological systems," "living organisms," or "derivatives thereof" (see Article 2).

Much of the synthetic biology research and most of its commercialised products (see Section 3.) involve the use of 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 in the following sections.

(a) *"Living modified organisms".*

The text of the Convention does not define "living modified organisms." According to the IUCN *Guide to the Convention*, negotiators 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 Cartagena Protocol's definition of living modified organisms, which applies to organisms obtained through the use of *modern* biotechnology, 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 Cartagena 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). 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". The areas of research that are considered "synthetic biology" but that are not "living" include DNA- and RNA-based circuits, protein engineering, metabolic pathway engineering, genome-level engineering, protocell construction, xenobiology, and cell-free systems as described in Section 2 above.

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

Both Articles 8(g) and 19, paragraph 4 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: (i) familiarity with the organism and its characteristics; (ii) the organism's contemplated application; and (iii) the environment into which the organism will or could be released (Glowka *et al.* 1994).

The Cartagena Protocol on Biosafety may also be relevant in this regard. As considered further in Section 8.2.2(a)., 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 Section 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) addresses "risks associated with the use and release" of living modified organisms. One possible interpretation of this text is that *two* categories of risks are included – risks associated with the use of living modified organisms and risks associated with the release of living modified organisms. The text could also be interpreted to consider only those risks associated with both the use *and* release of living modified organisms.

Most synthetic biology products that are commercially available have been intended for use in contained, industrial or laboratory settings (see Section 3.3.), for example for biopharma, carbon cycling, fabric, cosmetic/fragrances, 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 genome edited soya beans, rapeseed and engineered insects. Products intended for release are anticipated to increase significantly in coming years with field trials or near-market ready research spanning a broad range of applications, including genome edited animals and plants, genetically engineered nitrogen-fixing bacteria, engineered gene drives in mosquito for potential control of vector-borne diseases, engineered gene drive for an agricultural pest, products for bioremediation, biodegradation, biomining, and the transient modification of agricultural plants through RNA-based spray, amongst others as further elaborated in

Section 3. 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*[39].

Article 15 recognises 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.

Article 15 may be relevant to synthetic biology if it involves the access to genetic resources for use in synthetic biology processes. This would give rise to an obligation to share benefits from the utilisation of the genetic resources.

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 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 utilises 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 word "functional" introduces a dynamic element, 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

[39] 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.

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 & Jaspers, 2020; Oldham, 2004). In an increasing array of contexts, researchers utilise 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' was raised in 2016 during the thirteenth meeting of the Conference of the Parties to the CBD and in decision XIII/16, the COP 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 by the second meeting of the Parties to the Nagoya Protocol (decision NP-2/14).

At COP 14 in 2018, 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*" (para. 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.[40]

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[41]. 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.

(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

[40] MOP-3 to the Nagoya Protocol also adopted a decision on digital sequence information on genetic resources in which it requested the Open-ended Working Group on the Post-2020 Global Biodiversity Framework to submit the outcome of its deliberations for consideration by MOP-4, see decision NP-3/12.

[41] 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.

of the Convention. For example, taking into consideration some of the areas of research that are considered synthetic biology as identified in Section 2:

- DNA-based parts and devices, synthetic metabolic pathway engineering, and genome-level engineering – These areas of research involve designing and synthesising 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 would raise 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 16-19).

A number of COP decisions (e.g. COP Decisions XI/29, XII/2 B, XIII/23 B and 14/24) have sought to implement technical and scientific cooperation and technology transfer pursuant to Articles 16 to 19 of Convention. These have been implemented through various partnerships, programmes and initiatives, including Global Taxonomy Initiative, the Bio-Bridge Initiative, 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 (CBD, 2021). Further, pursuant to COP 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 Section 8.1.4 and Section 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: (i) be of relevance to conservation and sustainable use of biodiversity, and; (ii) 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 criteria, 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 Section 3.1.3. concerning research applications of synthetic biology in bioremediation, control of vector borne diseases for conservation purposes and improving resilience in wild animal and plant populations. Considering the second criteria, 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, holding that Parties "shall 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 they 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 Contracting Parties" (Article 19, paragraph 1, and Article 15, paragraph 6).

Scientific publications provide a useful proxy indicator for R&D concerning synthetic biology, by 2017, more than 25,000 authors at 3700 organisations located in 79 countries had contributed to synthetic biology research (Shapira et al., 2017). Since 1980, 13050 papers on synthetic biology have been published; with the USA, UK, Germany, China, and France leading the number of publications (French, 2019; Shapira et al., 2017). Other sites of major research include Japan, Switzerland, Italy, Spain, and Canada (French, 2019). A 2012 study which also evaluated scientific publications concerning synthetic biology depicts a similar global landscape with the exception of China which was then merely noted for its R&D activity alongside other emerging major economies, including Brazil, India, Mexico, Argentina, South Africa and Singapore (Oldham et al., 2012).

8.1.7. Provisions related to Indigenous Peoples and Local Communities (Articles 8(j) and 10(c)).

The preamble to the Convention recognises "*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 "*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 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 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 Section 5.1.2. concerning IPLC's specifically, and also Section 5.3. concerning ethical concerns more generally. Notably, these guidelines and other tools include:

- At its 7th meeting in 2004, the COP adopted 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 (Secretariat of the Convention on Biological Diversity, 2004).

- At its 10th meeting in 2010, the COP adopted the Code of Ethical Conduct on Respect for the Cultural and Intellectual Heritage of Indigenous and Local Communities Relevant for the Conservation and Sustainable Use of Biological Diversity (Secretariat of the Convention on Biological Diversity, 2011)
- At its 13th meeting in 2016, the COP adopted the Mo'otz Kuxtal Voluntary Guidelines 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 (Secretariat of the Convention on Biological Diversity, 2019).

More recently, at its 14th meeting in 2018, the COP 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 organisms 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 periodic expert working groups appointed by the Parties to evaluate issues related to synthetic biology under the Convention, as described further in Section 8.1.8. below. For example, the experts appointed to the AHTEG on Synthetic Biology convened in 2019 included two experts nominated by IPLC organisations. 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, as was appropriate communication and engagement with communities enable them to engage in the assessment of actual and potential impacts of synthetic biology. This built on an acknowledgement by the 2017 AHTEG that 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 IPLC's with Mother Nature, as well as noting that 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 decision by the COP referring directly to synthetic biology was adopted by COP 10 in 2010. Since then, the COP has adopted a number of decisions concerning synthetic biology as summarised in Table 1 below.

These incremental decisions have driven a steady build-up in intersessional activities focused on synthetic biology. COP 10 commenced a process of information gathering through invitations for the submission of information on synthetic biology which has been repeated for subsequent COP meetings, initially for the consideration by the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA) and subsequently to inform the deliberations the Ad Hoc Technical Advisory Group (AHTEG) on Synthetic Biology which was first established by the COP in Decision XII/24 and whose mandate has been extended at subsequent meetings. An open-ended online forum to support the work of the AHTEG has also been extended biennially. Synthetic biology-related documents such as submissions of information, AHTEG and

online forum reports are available through the online portal on Synthetic Biology[42]. 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 COP meetings. 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[43].

Table 1. Summary of COP 10-14 Decisions concerning Synthetic Biology.

Decision	Title	Key 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 living modified organisms 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	- Noting 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. - Recognising 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 & 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 apply take a precautionary approach, in accordance with paragraph 4 of decision XI/11 and 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 living modified organisms containing gene drives. - Acknowledged the operational definition of "synthetic biology" and considered it a 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 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,

[42] https://bch.cbd.int/synbio/

[43] Decisions BS-VII/12, XII/24, 14/19 and 9/13.

		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 COP-MOP to the Cartagena Protocol, 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 the COP-MOP to the Cartagena Protocol 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 organisms containing engineered gene drives into the environment when: ➢ Scientifically sound case-by-case risk assessments have been carried out; ➢ Risk management measures are in place to avoid or minimise 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.

8.2. Cartagena Protocol on Biosafety.

The Cartagena Protocol on Biosafety (Cartagena Protocol) applies to the transboundary movement, transit, handling and use of all living modified organisms (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). Article 1 of the Cartagena Protocol explicitly refers to the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development. The Cartagena Protocol entered into force in 2003 and has 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 Cartagena Protocol. Risk assessments undertaken pursuant to the Cartagena Protocol must be carried out in accordance with Annex III as specified in Article 15 Cartagena Protocol; 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 Cartagena 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). To be considered LMOs, the applications of synthetic biology would thus have to: i) be a living organism, ii) possess a novel combination of genetic material, and; iii) result from the use of modern biotechnology. It should be stressed that these terms are intrinsically interlinked, such that 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 Cartagena 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. 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 Section 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 "living modified organisms" 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 Cartagena Protocol. It agreed that this conclusion was still valid (Secretariat of the Convention on Biological Diversity, 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 recognised that the relevant obligations in the Convention would continue to apply (see also Section 8.1.4. above).

Examining the three elements of the definition of "living modified organism" in the Cartagena Protocol as outlined above can help to see how it relates to applications of synthetic biology.

(a) *Living organisms.*

The Cartagena 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). "Genetic material" is not defined in the Cartagena 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: i) products of organisms resulting from synthetic biology techniques; and ii) 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 Cartagena Protocol (Ascencio et al., 2003). "Products thereof" in the context of the Cartagena 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).

Organisms resulting from synthetic biology techniques that are currently used for commercial purposes are largely micro-organisms that have been altered to produce specific compounds, such as specialised 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. 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 (Ascencio 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 BioBrick Assembly Kit for sale over the internet. Components of the kit include

destination plasmids and the upstream and downstream parts as purified DNA[44]. Purified, synthetic DNA from commercial DNA synthesis firms is available in a lyophilised (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 may not qualify as "living organisms" under the Cartagena Protocol.

The Cartagena Protocol 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).

In practice, however, many countries do not apply the Cartagena 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.

- Novel combination.

A "novel combination of genetic material" 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 Horsepox virus Noyce et al. (2018), karyotype engineered yeast (Luo et al., 2018; Shao et al., (2018) and the JCVI-syn3.0 strain (Hutchison et al (2016) (see Sections 2.4 and 3.3.3) are not exact copies of the originals, and thus would qualify as novel. The use of directed evolution, multiplex automated genome engineering, genome editing techniques that do not incorporate new genetic material, would likely still be considered to result in 'novel combinations' because they could rearrange or extensively change existing genetic material, as in the case for gene shuffling (Magocha et al., 2018; Simon et al., 2019; Zhang et al., 2020).

(b) *Modern biotechnology.*

"Modern biotechnology" is defined in the Cartagena 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).

The negotiators of the Cartagena Protocol recognised that new techniques for modifying genetic information would continue to be developed (Ascencio et al., 2003). According to the IUCN explanatory guide, although the definition gives two specific examples of *in vitro* nucleic acid techniques, other techniques 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 Cartagena 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. Others are of the impression that genome editing techniques are not

[44] https://www.neb.com/products/e0546-biobrick-assembly-kit#Product%20Information

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).

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

The Cartagena 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). This Section provides an overview of the Protocol 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 Cartagena Protocol.

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

Under Article 15, paragraph 2, 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 to proceed (Article 10 and Article 15, paragraph 2, Cartagena Protocol). Risk assessments must be "*carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognised risk assessment techniques*" (Article 15, paragraph 1 Cartagena Protocol). A risk assessment as per Annex III is also required if a developing country Party or a Party with an economy in transition that does not have a domestic regulatory framework decides to import an LMO-FFP and has indicated that its decision prior to import will be taken on this basis (Article 11, paragraph 6(a) Cartagena Protocol).

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.

Annex III of the Cartagena Protocol provides general principles, methodology, and points to consider in a risk assessment (see also Section 6.1). Paragraph 8 of Annex III outlines a number of steps to meet this objective, providing that a risk assessment entails, as appropriate, the following steps:

- An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;
- An evaluation of the likelihood of these adverse effects being realised, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;
- An evaluation of the consequences should these adverse effects be realised;
- An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realised;
- A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks; and
- Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment.

The risk assessment may take into account the characteristics of the recipient organisms, donor organisms, receiving environment, the introduced modification, and the identity of the LMO (Annex III, paragraph 9, Cartagena Protocol). The Parties have also developed voluntary guidance on risk assessment of living modified organisms including a roadmap for risk assessment of LMOs that supplements Annex III of the

Protocol, as well as guidance on the risk assessment of specific types of LMOs and traits as well as the monitoring of LMOs released into the environment. First developed in 2012 the voluntary guidance was updated and in 2016 the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety invited interested Parties, other Governments and relevant organizations to take the Guidance[45] into account as a voluntary tool to assist in conducting risk assessment (Decision VIII/12).

Although LMOs produced through synthetic biology may present characteristics that are not common to all LMOs, Annex III of the Protocol, including its general principles, points to consider and methodology are still fully applicable to living organisms 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). 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 optimised. In these cases, there might not be an appropriate comparator.

In Decision BS-II/9 in 2008 the Parties considered risk assessment and risk management and established an Ad Hoc Technical Expert Group on Risk Assessment (AHTEG-RARM) to further consider and evaluate the nature and scope of existing approaches to risk assessment and identify existing gaps, and capacity-building needs. It also established an open-ended online forum, through the Biosafety Clearing-House, to assist the AHTEG-RARM. In 2012 and in 2014, the AHTEG-RARM identified the risk assessment of LMOs produced through synthetic biology among a set of topics for the development of further guidance. In Decision BS-VIII/12 in 2016, the COP-MOP acknowledged the completion of the mandate of the AHTEG-RARM.

More recently in Decision CP-9/13 in 2018, the Conference of Parties serving the meeting of Parties to the Cartagena Protocol on Biosafety (COP-MOP) recognised the divergence in views among Parties on whether or not additional guidance on specific topics of risk assessment is needed. The COP-MOP noted the conclusions of the AHTEG on Synthetic Biology concerning, *inter alia*, the uncertainties regarding engineered gene drives. It decided to establish a process for the identification and prioritisation 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 living modified organisms containing engineered gene drives and living modified fish. 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 living modified organisms are to be considered by the Subsidiary Body on Scientific, Technical and Technological Advice with a view to enabling the Subsidiary Body to prepare 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 10th meeting. At the time of writing, the meetings of the Subsidiary Body on Scientific, Technical and Technological Advice, the Subsidiary Body and the Conference of the Parties, were still to be held.

(b) *Advanced Informed Agreement 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 a 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.

[45] The Guidance on Risk Assessment of Living Modified Organisms and Monitoring in the Context of Risk Assessment is available at https://www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf.

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. There are limited exemptions to the requirements of the Advance Informed Agreement procedure, as follows:

- "Contained use" (Article 6).

  Under the Cartagena 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)[46]. Contained use is defined as an operation, "undertaken within a facility, installation or other physical structure," in which the LMOs' contact with and impact on the external environment is "effectively limit(ed)" by "specific measures" (Article 3(b); Cartagena Protocol). Negotiations on this topic concentrated on whether chemical or biological barriers could be considered as sufficient containment, or whether physical containment was necessary (Ascencio 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 (Ascencio et al., 2003). In decision CP-9/12, the MOP reminded Parties 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.

  At least three issues have been raised by some civil society groups in relation to synthetic biology and the "contained use" AIA exemption. First, 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[47]. Importing countries may need advance information in order to "judge the effectiveness of available containment" (*Ibid*). See also Section 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, has raised doubts as to whether DIYbio (do-it-yourself biology) individuals and collectives can ever be considered a "contained use" operation (EcoNexus, 2011). EcoNexus does not consider "garage biotech facilities" as contained use and is concerned that AIA "might become close to impossible" in such instances (EcoNexus, 2011). A Woodrow Wilson International Center for Scholars report on DIYbio found that 92 % of DIYers work in group spaces (not alone), that few DIYers are using "sophisticated" synthetic biology, and most work in labs that are rated as Biological Safety Level 1 (Grushkin et al., 2013). Considering the current status of the synthetic biology practiced by DIYers, the Woodrow Wilson International Center for Scholars report finds that DIYers present a low risk to the environment. It does, however, note that future boundaries between home and group labs may be porous, leading to experiments being carried in transit and possibly spilling, and issues around the disposal of lab waste (Grushkin et al. 2013). These are issues around contained use, although again, Grushkin *et al.* (2013) see this as possible future concerns depending on the development of synthetic biology and the DIYbio communities. Others have similarly concluded that DIYBio has so far been a responsible and transparent citizen science movement (Landrain et al., 2013; Seyfried et al., 2014). More recently developments involving self-regulation by the scientific community which are relevant to the DIYbio discussion are considered in Section 7.3.

[46] The Cartagena Protocol 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 (Ascencio et al., 2003).

[47] 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 analogs in the natural world (ICSWGSB 2011).

A third and more general issue, which is not limited to LMOs produced by synthetic biology, is that Parties could be faced with "regulatory arbitrage" − the practice of utilising more favourable laws in a jurisdiction to circumvent regulation elsewhere − if a laboratory imports a synthetic biology LMO for contained use and then makes a domestic application to release the synthetic biology LMO from containment (ICSWGSB 2011). This could have adverse implications for biosafety, for example, if domestic standards for risk assessment may be lower than the minimums provided in the Cartagena Protocol's Annex III. Examples of regulatory arbitrage concerning synthetic biology are not readily apparent, however, concerns have been expressed that diverging regulatory or ethical standards could increase the risk of the creation of potentially harmful biological agents or products (Gronvall, 2018). Concerns regarding dual-use are considered in Section 5.4.

- 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). 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 of the Protocol (Article 11, paragraph 1 and Annex II (j); Cartagena Protocol). 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).

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

The Cartagena 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). 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, horsepox and adenovirus vector for coronavirus and SARS-CoV-2 vaccines (Forni & Mantovani, 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 Ascencio et al., 2003). Vaccines produced using synthetic biology techniques, however, would likely be considered pharmaceuticals under Article 5 of the Cartagena Protocol[48]. 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 organisations to be exempted from the Cartagena Protocol. It is unclear to what extent LMOs that are pharmaceuticals for humans would need to be "addressed" by other international agreement or organisation to qualify for the Article 5 exemption. In particular, it is an open question whether the agreement or organisation must address the biodiversity impacts of the LMO (Ascencio et al., 2003).

[48] The IUCN Guide to the Cartagena Protocol reports that living modified organisms that are pharmaceuticals for humans are "principally genetically engineered vaccines" (Mackenzie et al. 2003). In comments to an earlier version of this document, one organization noted that "continued research and development of vaccines, whether for humans or animals, may be discouraged if synthetic biology is further included within the Cartagena Protocol."

Currently, none of the organisms produced through synthetic biology that are intended to be used as pharmaceuticals for humans are directly addressed by other relevant international agreements or organisations. For example, a commonly invoked promise of synthetic biology is the rapid development of vaccines using viruses (Dolgin, 2020; PCSBI, 2010; RAE, 2009). Therefore, such living organisms would fall under the Cartagena Protocol's scope.

8.2.3. Other relevant provisions of the Protocol.

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 deals with the unintentional transboundary movements and emergency measures in order to avoid or minimise risks to biodiversity and human health within the jurisdiction of other States. Where an unintentional transboundary movement occurs within the jurisdiction of a Party, the Party is under an obligation to notify any affected or potentially affected States, the Biosafety Clearing-House and international organisations where appropriate. Where the affected or potentially affected State is not a Party to the Cartagena Protocol, it must be notified in line with principles of international customary laws. 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 the light of the nature and scale of the transboundary movement in question and the possible adverse effects on biodiversity and human health (Ascencio et al., 2003).

(b) *Illegal transboundary movements.*

Article 25 of the Cartagena 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) penalise transboundary movements of LMOs which contravene its national measures to implement the Protocol (Ascencio et al., 2003). A Party affected by an illegal transboundary movement of LMOs to 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 socio-economic considerations.*

In reaching decisions on imports Article 10 of the Protocol requires Parties to evaluate potential impact of the LMO concerned on the conservation and sustainable use of biological diversity, taking into account risks to human health. Article 26 of the Protocol addresses the extent to which Parties are entitled to take socio-economic considerations into account in reaching a decision on imports of LMOs, including the value of biological diversity to IPLCs. Additionally, Article 26 encourages Parties to cooperate on research and information exchange on any socio-economic impacts of living modified organisms, especially on IPLCs.

(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 in order to ensure effective implementation of the Protocol by developing country Parties. Least developed countries and countries with economies in transition are prioritised to ensure they have adequate human, technical and financial resources to implement appropriate biosafety measures and standards: for example, to undertake risk assessment and risk management of LMOs, or to monitor LMOs once released into the environment (Ascencio et al., 2003). 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 Section 8.1.6.

(e) *Handling, transport, packaging and identification*.

Article 18 requires Parties to take measures for the safe handling, packaging and transport of LMOs that undergo intentional transboundary movement in order to minimise risks to biodiversity and human health. This applies to all LMOs within the scope of the Protocol, whether or not they are subject to the specific AIA procedure described in Section 8.2.2(b) (Ascencio et al., 2003). It 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 (Ascencio 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 Biosafety Protocol. 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 Protocol (Article 27). The result of this process was the Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol (Supplementary Protocol), which was adopted by the Conference of the Parties serving as the meeting to the Parties to the Cartagena Protocol at its fifth meeting in 2010. The Supplementary Protocol entered into force on 5 March 2018 and has 48 Parties as of March 2021.

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 living modified organisms.

This Supplementary Protocol applies to damage resulting from LMOs which find their origin in a transboundary movement and are (i) intended for direct use as food, feed, or for processing; (ii) destined for contained use; or (iii) intended for intentional introduction into the environment (Article 3; Supplementary Protocol). It applies to damage resulting from any authorised use of the LMOs, damage resulting from unintentional transboundary movements as referred to in Article 17 of the Cartagena Protocol, as well as damage resulting from illegal transboundary movements as referred to in Article 25 of the Cartagena Protocol.

"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 recognised by a competent authority that takes into account any other human induced variation and natural variation; and is significant. Whether an adverse effect is "significant" is to be determined on the basis of factors, such as:

(i) the long-term or permanent change, to be understood as change that will not be redressed through natural recovery within a reasonable period of time;

(ii) the extent of the qualitative or quantitative changes that adversely affect the components of biological diversity;

(iii) the reduction of the ability of components of biological diversity to provide goods and services; and

(iv) the extent of any adverse effects on human health in the context of the Protocol (Article 2, paragraph 3).

A causal link needs to be established between the damage and the LMO in question in accordance with domestic law (Article 4; Supplementary Protocol). In the event of damage, the Supplementary Protocol provides for an administrative approach by which operators are required to immediately inform the competent authority, evaluate the damage; and take appropriate response measures (Article 5(1); Supplementary Protocol). Furthermore, where relevant information indicates that there is a sufficient likelihood that damage will result if timely response measures are not taken, the operator shall be required to take appropriate response measures so as to avoid such damage (Article 5(3)).

The terms "operator" and "response measures" are both defined in the Supplementary Protocol. An operator refers to a person in direct or indirect control of LMO, as may be determined by domestic law, including *inter alia*, the permit-holder, person who placed the living modified organism on the market, developer, producer, notifier, exporter, importer, carrier or supplier. Response measures refer to reasonable actions to prevent, minimize, contain, mitigate or otherwise avoid damage, as appropriate, or reasonable actions to restore biological diversity.

The Supplementary Protocol also addresses civil liability for material or personal damage associated with the damage, as defined in the Supplementary Protocol, and enables Parties to continue to apply their existing law on civil liability and/or develop and apply civil liability law specifically for this purpose (Article 12(2)).

As discussed in Section 8.2.1. above, organisms resulting from synthetic biology techniques may fall under the definition of a "living modified organism" under the Cartagena Protocol. Further, as described in Section 4 of this document, it is possible that LMOs resulting from synthetic biology techniques could cause adverse effects on the conservation and sustainable use of biological diversity. For example, unintentionally released organisms may transfer the inserted genetic material and thus change biodiversity at a genetic level, intentionally released organisms may become invasive due to engineered fitness advantages. Such concerns are particularly acute in the context of engineered gene drives in which the environmental release of such organisms relates to the targeting of wild populations and may be irreversible, as considered further in Sections 4.1. and 6.1.1. As has been discussed, there appears to be significant controversy as to the scope and therefore "significance" of the potential damages. The applicability of the provisions of the Supplementary Protocol would have to be assessed for particular cases.

8.4. 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 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 has 130 Parties as of March 2021[49].

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.

[49] See http://www.cbd.int/abs/nagoya-protocol/signatories/default.shtml.

The following examines additional 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 in the Protocol. It provides that the terms defined in Articles 2 of the Convention also apply to the Protocol and consequently, discussions on the definitions of "genetic resources" and "genetic material" in Section 8.1.5. are also relevant for this chapter. It defines "utilization of genetic resources" as conducting 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 Protocol means any technological application that uses 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 food and feed are under advance development or commercially available in the agricultural sector. If used *solely* as a feedstock, this use of sugarcane would likely not fall within the "utilization of genetic resources." However, if research was conducted on the sugarcane to determine if it was an appropriate feedstock or if it could be transformed to be more suitable, this research could be interpreted as "utilization" within the terms of the Nagoya Protocol, and access to the sugarcane 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 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).

The use of these synthetic biology techniques raises questions as regards to until what extent the results of modifications of a natural genetic resource continue to be subject to the benefit-sharing obligations. Article 5, paragraph 1 of the Nagoya Protocol requires that benefits arising from the utilisation of genetic resources "*as well as subsequent applications and commercialization*" shall be shared in a fair and equitable way. It also provides that "such sharing shall be upon mutually agreed terms". According to Greiber, this is meant to extend benefit-sharing to processes and products developed along the value chain (Ahrén et al., 2012).

National implementation and the negotiation of mutually agreed terms can assist Parties to an access and benefit-sharing agreement to clarify until which extent of 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 Section 8.1.5., discussions are ongoing under the CBD 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[50].

The Nagoya Protocol in its Article 2 defines a "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 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 synthesised organisms could be considered a "derivative" as defined by the Protocol.

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 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 optimised for expression in engineered microorganisms" (Erickson et al., 2011).

An initial question is whether genetic resources from *H. brasiliensis* were actually accessed and "utilized" in the context of the Protocol. A separate question might be whether access to derivatives of organisms resulting from synthetic biology techniques – such as isoprene – would also be covered by the Nagoya Protocol (see similar discussion on access to genetic resources originating from synthetic biology in Section 8.1.5.)

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 utilisation of genetic resources and biotechnology (Article 2; Nagoya Protocol, see Ahrén et al., 2012; Singh Nijar, 2011). Another possible interpretation is that the operative provisions of the Protocol apply only to genetic resources, and not to derivatives[51].

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 until which extent of 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 Rules and Regulatory Practices, 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 laws, processes and initiatives beyond the Convention and its Protocols are anticipated to shape the governance of synthetic biology. This section considers other international laws, processes and initiatives with potential implications for the governance of synthetic biology which are relevant to the work of the CBD, as summarised in Table 2 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

[50] 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.

[51] See Singh Nijar (2011) for descriptions of the arguments for differing interpretations of the role of derivatives in the Nagoya Protocol.

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 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 analysis is available concerning potential gaps in international governance. Additionally, this update prioritises coverage of initiatives or organisations 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 B, this Section cannot be construed as an exhaustive coverage of international laws, processes and initiatives which have potential implications for the governance of synthetic biology.

Table 2. International rules, regulatory practices, processes, and initiatives with potential implications on the governance of synthetic biology related to the work of the CBD

International rules, processes, and initiatives	***Conservation***	***Sustainable use***	***ABS***	***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	x	x		Risk (health)
Environmental Modification Convention (ENMOD)				Risk (environment)
UN Declaration on the Rights of Indigenous Peoples	x	x	x	
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	x	x	x	Food security
The United Nations Convention on the Law of the Sea (UNCLOS) proposed agreement for marine biodiversity beyond national jurisdiction (BBNJ)	x	x	x	Marine GR in ABNJ
WTO Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS)		x		IP
International Convention for the Protection of New Varieties of Plants (UPOV Convention)		x		IP
The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) *The International Plant Protection Convention (IPPC)*		x		Trade; Risks (phyto-sanitary)

World Organization for Animal Health		x		Trade
Codex Alimentarius		x		Trade

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

9.2.1. World Health Organization (WHO).

The World Health Organization is a specialised 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 (WHA) as WHO's governing body which meets annually. Various areas of work within this organisation could be related or have an impact on synthetic biology governance. These areas are described below.

(a) *Responsible Life Sciences Research.*

In 2010, the WHO published "Responsible Life Sciences Research for Global Health Security" as a Guidance Document[52] which reviewed the types of life sciences research that may be of concern, offering several examples, including synthetic recreation of viral genetic material. The document outlined a range a range of complementary policy options for managing potential risks, including the following which 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, policy-makers and the public. It also proposed a 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 2021, the WHO is undertaking a review of the terminology around responsible life sciences research and dual use research of concern with a view to updating guidance in this area of work, particular in light of biomedical advances since 2010.

(b) *International Health Regulations, pandemic preparedness and Biorisk management*

In the wake of the COVID-19 pandemic, the WHO is undertaking a comprehensive review of the functioning of the International Health Regulations (2005)[53], 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, the WHO and UNODA have teamed up as co-leads on a new United Nations internal system-wide Biorisk Working Group established by the Secretary-General. The United Nations Biorisk Working Group aims at bringing together policy/normative and technical expertise to further develop a clear understanding of capacities, mechanisms, roles and responsibilities, and harmonise them within the UN system. This will strengthen the international community's preparedness and response to natural, accidental or deliberate biological events[54].

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

[53] https://www.who.int/teams/ihr/ihr-review-committees/covid-19

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

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

At the Sixty-seventh World Health Assembly in May 2014, the 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 (IAG) 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) on Synthetic Biology and Variola Virus and Smallpox was convened in April 2015 to provide the technical and scientific background for the IAG.

As noted in the IAG's report (WHO, 2015) the conclusions of the SWG were as follows:

> *"With the rapid advances in synthetic biology, there is now the capability to recreate the variola virus, the causative agent of smallpox. While recreating variola is quite complex, it is increasingly possible due to the availability of genetic material and of machines for complex assembly, as well as increasing know-how among a broad array of persons. Furthermore, the rapid rise in availability of genetic material from commercial sources and the so-called "grey market" is driving the cost of this material down, making recreation possible by multiple institutions and persons, including those with malicious intent. The "WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA" should be revised. consideration should be given to adding a component or separate document on guidance to commercial DNA providers for screening requests for DNA fragments. With the development of these technologies, public health agencies have to be aware that henceforth there will always be the potential to recreate variola virus, and therefore the risk of smallpox re-emerging can never be fully eradicated."*

The IAG concluded that the risk of the re-emergence of smallpox overall has increased. They recognised that the creation of the variola virus, using information on DNA sequences, would be easier and cheaper in the future, and may be possible in small laboratories that have inadequate biosafety and biosecurity for handling the virus. It therefore recommended the following to the WHO[55]:

- to increase significantly preparedness efforts to ensure that early detection and rapid response capacities, including those in risk communications, for a potential smallpox re-emergence are widely available; and
- to revise the WHO regulations for the handling of variola virus (whole virus or fragments) with particular emphasis on biosafety and biosecurity rules and regulations to reduce and minimise the risk of a laboratory accident that may occur from the widespread use of synthetic biology technology.

Following these synthetic biology consultations, the WHO sought input from the Advisory Committee for Variola Virus Research and in 2016 updated its recommendations concerning the distribution, handling and synthesis of variola virus DNA[56]. It contains the following guidance on the synthesis of variola virus DNA:

- Attempts to synthesise full-length variola virus genomes or infectious variola viruses from smaller DNA fragments are strictly forbidden.
- Synthesis of variola virus DNA to express a variola virus protein, or synthesis of codon modified DNAs for the same purpose requires prior permission from the WHO. Similarly, mutagenesis of orthopoxvirus DNA with the aim of producing a variola virus protein requires prior permission from the WHO through

[55] WHO, Synthetic biology technologies, June 2015 (https://www.who.int/news-room/feature-stories/detail/synthetic-biology-technologies)

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

the same channel. Those undertaking synthesis of variola virus DNA are under the same obligations and constraints outlined above in the section on distribution of variola virus DNA.

- Under no circumstances can any single laboratory other than the designated WHO Collaborating Centres hosting the variola virus repositories hold DNA comprising more than 20 % of the total genome, except that permission from the WHO is not required for the production of DNA microarrays, on which small oligonucleotides (less than 80 base pairs) are covalently bound to a matrix and which, in aggregate, may span the entire variola virus genome.
- For diagnostic kit purposes, variola virus DNA fragments up to 500 nucleotides may be synthesised without notification to the WHO.
- These policies also extend to the manufacturers of synthetic DNA who must also be held responsible for upholding these recommendations and national policies.

The WHO 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, the 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)[57]. 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 the WHO's position are summarised 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 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.*

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

- *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."*

The 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."*

(e) *WHO Laboratory Biosafety Manual*[58].

The WHO released a 4th edition of its WHO Laboratory Biosafety Manual in December 2020 (WHO, 2020b). 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. 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. The 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 optimised 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.

Synthetic biology is recognised in Section 8.8. as an emerging technology (alongside genetically modified microorganisms, synthetic biology, 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 policy-making, and public trust and confidence in science. However, countries, laboratories and scientists must also consider the risks posed by incidents and/or the potential deliberate misuse of life sciences research and select appropriate control measures to minimise those risks in order to conduct necessary and beneficial life sciences research.

The Manual is comprised of a core document and subject-specific monographs providing guidance on the following:

- Risk assessment
- Laboratory design and maintenance
- Biological safety cabinets and other primary containment devices
- Personal protective equipment
- Decontamination and waste management
- Biosafety programme management

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

- Outbreak preparedness and resilience

(f) *Pandemic Influenza Preparedness (PIP) Framework.*

The WHO's PIP Framework was unanimously adopted by the Sixty-fourth World Health Assembly in 2011. It brings together Member States, industry, other stakeholders and WHO to implement a global approach to pandemic influenza preparedness and response with the key objective to improve and strengthen the sharing of influenza viruses with human pandemic potential; and to increase the access of developing countries to vaccines and other pandemic related supplies.

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: to improve and strengthen 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[59] and to increase the access of developing countries to vaccines and other pandemic related supplies. The Framework was developed by Member States. It came into effect on 24 May 2011 when it was unanimously adopted by the Sixty-fourth World Health Assembly (2011)[60].

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

- Partnership Contribution - An annual cash contribution of US$ 28 million 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 vaccine 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 Global Influenza Surveillance and Response System (GISRS).
- Virus Sharing - Influenza virus sharing, conducted by the WHO Global Influenza Surveillance and Response System (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.

PIP Framework is the only pathogen-specific international access and benefit-sharing (ABS) instrument. Similarly to the 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 Section 9.3.3.(a). regarding the ITPGRFA).

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

[59] https://www.who.int/influenza/pip/virus_sharing/en/

[60] https://www.who.int/influenza/pip/en/

international trade in specimens of wild animals and plants does not threaten their survival. CITES currently has 183 Parties (as of March 2021) 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 17th meeting (CoP17, in 2016), the Conference of the Parties requested the Secretariat[61] 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[62]
- under what circumstances wildlife products produced from synthetic or cultured DNA meet the current interpretation of such resolutions; and
- 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 Secretariat commissioned a Study on Wildlife Products Produced from Synthetic or Cultured DNA[63] 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[64]. At its 69th 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 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 recognisable,' 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 70th 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

[61] Pursuant to Decisions 17.89 to 17.91 available at https://cites.org/sites/default/files/eng/com/sc/69/E-SC69-35.pdf.

[62] Specifically pursuant to Resolution Conf. 9.6 (Rev. CoP16) concerning Trade in readily recognisable parts and derivatives, available at https://old.cites.org/eng/res/09/09-06R16.php.

[63] The study is available as Annex 6 to Document SC70 33 available at https://cites.org/sites/default/files/eng/com/sc/70/E-SC70-33-A6.pdf.

[64] As noted in the Meeting document CoP18 Doc. 43 'Specimens Produced from Synthetic or Cultured DNA' submitted to Eighteenth meeting of the Conference of the Parties, 23 May – 3 June 2019.

recommendations prematurely. The meeting record indicates some Committee Members and Parties expressed as to whether or not specimens produced by biotechnology fell under the remit of CITES[65].

The Standing Committee's recommendations were considered at CoP18 in 2019 and COP Decision 18 regarding "specimens produced through biotechnology"[66] included the following directions[67]:

- Parties were invited to provide information regarding: (i) cases where they have issued, or received requests to issue, CITES permits and certificates for specimens produced through biotechnology; (ii) other situations when they have applied the interpretation of Resolution Conf. 9.6 (Rev. CoP16) on Trade in readily recognisable parts and derivatives to fauna and flora products produced through biotechnology; and (iii) technological developments and applications taking place, particularly in their 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: (i) make recommendations, including appropriate revisions to existing resolutions; and (ii) provide any relevant scientific advice and guidance on matters relevant to international trade in specimens produced through biotechnology.
- The Secretariat was requested to coordinate with the Secretariats 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.

International Union for Conservation of Nature (IUCN) is a membership Union composed of governments and civil society organisations with a focus on nature conservation and sustainable development. It has more than 1,400 member organisations 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 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 and its Protocols and most other international initiatives considered in this update, 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 –

[65] As per the Summary Record Seventieth meeting of the Standing Committee SC70SR available at https://cites.org/sites/default/files/eng/com/sc/70/exsum/E-SC70-SR.pdf.

[66] 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.

[67] As per Decisions 18.147 - 18.150 Specimens produced through biotechnology available at https://cites.org/eng/taxonomy/term/42062.

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 the biodiversity conservation in relation to synthetic biology. A series of IUCN principles on synthetic biology and biodiversity conservation were proposed in a Council motion submitted for the 2020 IUCN World Conservation Congress that was initially scheduled to take place in June of 2020. This motion was discussed by the IUCN membership during an online discussion held from December 2019 until March 2020. The motion is still open for further discussion during the formal session of the IUCN Congress - currently scheduled to take place in September 2021 - before being put to the vote of the IUCN membership at the Members' Assembly.

The technical assessment includes detailed coverage of synthetic biology applications which are directly or indirectly intended for conservation benefit, governance challenges raised by synthetic biology conservation, and an evaluation of governance frameworks relevant to synthetic biology impacts on biodiversity. It also contains detailed case studies of synthetic biology applications, particularly engineered gene drives. Additionally, the adoption by IUCN Members of a series of principles that would serve as the basis for an IUCN policy thus providing guidance on synthetic biology and biodiversity conservation would likely have a significant influence on deliberations within the Convention and its Protocols, particularly by the various fora involving technical experts advising in relation to risk management, synthetic biology and digital sequence information on genetic resources, as described in Section 8.

9.3. Other International laws, processes, and initiatives with potential implications for the governance of synthetic biology.

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 regardless of whether they are a Party to, and bound by, a particular treaty (except for so-called "persistent objectors"). 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 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 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, which for the most part reflect customary law (Annex to UNGA Res. A/RES/56/83 of 12.12.2001, "Articles on State Responsibility")[68].

The rules on State responsibility do not define obligations relating to synthetic biology in the sense that they determine 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[69].

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 recognised 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.

Synthetic biology techniques may be conducted by both State-governed and private entities. The customary international law of 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

[68] The rules relevant to the present note 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.

[69] 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/66/10, paragraph 66, in particular principle 4.2. In contrast to many of the Articles on State Responsibility, these draft articles do not reflect customary law.

present (e.g., a private actor exercising elements of governmental authority)[70]. 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 nonstate 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, 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"[71]. In the *Pulp Mills* case, the Court used a slightly different wording[72]: "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"[73].

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[74].

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 the wording used by the ICJ in the *Pulp Mills* case, it appears that

[70] As per the relationships outlined in the Draft Articles on Prevention of Transboundary Harm from Hazardous Activities, as explained in footnote 64.

[71] 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.

[72] 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).

[73] ICJ, Case concerning Pulp Mills on the River Uruguay (Argentia v. Uruguay), ICJ Reports 2010, 14, paragraph 101.

[74] 31 ILM 876 (1992); cf. principle 21 of the preceding 1972 Declaration of the UN Conference on the Human Environment (Stockholm Declaration), 11 ILM 1416 (1972).

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 concerning state responsibility and liability of private actors immediately above).

Many synthetic biology research and commercial applications have the potential for transboundary impacts through economic, social, and cultural 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-localised) and persist indefinitely (self-sustaining), or be restricted in spread (localised) or persistence (self-limiting). Direct impacts on the transboundary environment, however, would depend on the specific application of synthetic biology. Currently, intentional environmental release of organisms resulting from synthetic biology techniques seem to be limited to a few instances such as commercially available soya bean engineered to obtain a high-oleic oil and engineered insects which contain a self-limiting gene resulting in either a reduction in the pest insect population that spread disease[75]. Anticipated applications of synthetic biology include the production of micro-organisms specifically designed for environmental release, such as for bioremediation of ocean oil spills (see Section 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 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. "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[76].

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.

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[77]. 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 which measures States are required to take in order to prevent such harm. Generally, a State will not be in breach of the obligation relevant here unless it fails to apply due diligence[78]. What diligence is "due", however,

[75] See section 4.2. above and also section 4.2.5. of the information document UNEP/CBD/COP/12/INF/11prepared by the Executive Secretary and submitted to the 12th meeting of the Conference of the Parties

[76] 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.

[77] 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).

[78] 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.

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[79]. 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 recognised that the accepted practice amongst 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"[80].

As discussed immediately 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 amongst 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 and that sufficient knowledge is available to characterise 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 risk assessment and risk management associated with genetically modified insects containing engineered gene drives (Naegeli et al., 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 USD 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

[79] 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.

[80] ICJ, Case concerning Pulp Mills on the River Uruguay (Argentina v. Uruguay), ICJ Reports 2010, paragraphs 204 -206.

drives, is one example of safety research, however, it has been noted that there is room for more of such efforts and that to reduce safety concerns in synthetic biology, more prominent support and funding is required regarding research into improvements in safety (Gronvall, 2018).

Significant adverse impacts that may occur include 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 analyses the extent to which existential risks have been discussed at an international governance level, specifically in documents in the UN Digital Library, argues 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, a precautionary approach has been introduced in the preamble recognising 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 minimise such a threat". Similarly, the objective of the Cartagena Protocol makes reference to 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 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). Multiple COP 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 1 above).

(b) *Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction.*

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 168 Parties. This agreement may apply to the use of components,

organisms and products resulting from synthetic biology techniques for hostile purposes or in armed conflict[81].

- Overview of main provisions.

The core provision of the Biological Weapons Convention is its Article I in which each Party to this Convention undertakes never in any circumstance to develop, produce, stockpile or otherwise acquire or retain: (i) 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 (ii) 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 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[82].

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" (BWC 1986).

In 2016, the Eighth Review Conference adopted a final declaration covering the full scope of the Convention which re-iterated "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

[81] Relevant in this context is also the Australia Group, an informal forum of countries which, through the harmonisation of export controls, seeks to ensure that exports do not contribute to the development of chemical or biological weapons. The 41 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 programs. Since 2007, meetings of the Australia Group have discussed synthetic biology, see www.australiagroup.net.

[82] A Review Conference is a conference of State Parties, which, in accordance with Article XII of the Convention reviews the operation of the Convention and also considers, among others, new scientific and technological developments relevant to the Convention.

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" (BWC, 2017). 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 immunisation, 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 (Goldblast, 1997). 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.

- Inter-sessional review of developments in the field of science and technology.

Implementation of the Convention 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.[83] The 2017 Meeting of the State Parties approved the following developments in the field of science and technology for review at the annual Meetings of Experts in the period 2018-2020 (BWC, 2017)[84]:

- 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 the WHO, OIE, FAO, IPPC and OPCW.

The common understanding reached by States Parties on these topics will be reported at the 2020 meeting of States Parties under the Convention which has been postponed to 22 to 25 November 2021, at which time the intersessional program is also anticipated to be updated[85]. The Ninth Review Conference is also anticipated to take place in 2021. To date, few discernible steps towards the development of an oversight framework, guiding principles, or models to inform risk are apparent, however, certain State 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[86].

[83] For references to working documents under the Biological Weapons Convention that address synthetic biology, see UNICRI 2011.

[84] https://undocs.org/bwc/msp/2018/mx.2/2

[85] https://meetings.unoda.org/meeting/bwc-msp-2020/

[86] For example, a submission by the US to the 2020 Meeting of Experts 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.

(c) *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 State Parties. ENMOD contains a mechanism for a conference of State Parties to be held every 5-10 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[87].

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 in 2013 and again in 2014[88] the Secretary-General of the United Nations initiated a process of soliciting the views of the States parties to convene a Third Review however, the minimum threshold does not appear to have been reached.

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 has clear implications for the use of synthetic biology in climate or weather modification.

9.3.2. Free, Prior and Informed Consent of Indigenous People and Local Communities.

(a) *Indigenous and Tribal Peoples Convention, 1989 (No. 169).*

The Indigenous and Tribal Peoples Convention, 1989 (No. 169) is an International Labour Organization Convention (also known as ILO-Convention 169) which as of March 2021 has 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.

(b) *UN 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 people worldwide date back over several decades, including a Working Group on Indigenous Populations convened 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[89].

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.

[87] https://www.un.org/disarmament/enmod/

[88] https://geneva-s3.unoda.org/static-unoda-site/pages/templates/enmod/UNSG%2BNV%2Bre%2BENMOD.pdf

[89] In 2006, the United Nations Commission on Human Rights was replaced by the United Nations Human Rights Council.

FPIC 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. The Article 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[90].

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 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 specialised agencies, as well as States shall promote respect for and full application of the provisions of UNDRIP and follow up its effectiveness.

9.3.3. Access & Benefit Sharing.

(a) *International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).*

- Overview of main provisions.

Article 2 of the 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, which defines genetic resources as genetic material of actual or potential value, and genetic material as any material of plant, animal, microbial or other origin containing functional units of heredity (Article 2). The main difference between the two treaties is that the definitions under the ITPGRFA only refer 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, on potential impacts, agricultural applications of synthetic biology are a focus of current research, as is the production of specialised plant feedstocks for bioenergy purposes. According to the IUCN explanatory guide to the ITPGRFA, the treaty text is ambiguous in whether functional units of heredity are in themselves plant genetic resources for food and agriculture (PGRFA) or are components of PGRFA (Moore & Tymowski, 2005). Thus, if synthetic biology research is based upon DNA sequences of PGRFA, it may be a matter of interpretation whether the research is utilising PGRFA.

According to Article 5 of the 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:

- Promote 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;
- Promote *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;
- Cooperate to promote the development of an efficient and sustainable system of *ex situ* conservation, giving due attention to the need for adequate documentation, characterisation, regeneration and

[90] Human Rights Council, Study of the Expert Mechanism on the Rights of Indigenous Peoples on Free, prior and informed consent: a human rights-based approach, 10 August 2018, (A/HRC/39/62).

evaluation, and promote 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; and

- Monitor the maintenance of the viability, degree of variation, and the genetic integrity of collections of plant genetic resources for food and agriculture; and
- Take steps to minimise 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 the 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 (see section 4). Also, some synthetic biology research is focused on modifying micro-organisms to produce substances that would substitute for Annex I crops, such as lauric acids that are currently produced in part from coconuts (see section 4)

Article 12 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 utilisation 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; and
- 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 commercialisation.

The latter is achieved through a requirement in the Material Transfer Agreement that a recipient who commercialises 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 commercialisation 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 commercialises shall be encouraged to make such payment.

While the Multilateral System applies only to the plant genetic resources for food and agriculture set out in Annex I to ITPGRFA, genetic resources not listed in Annex I and held by the International Agricultural Centres and other international institutions, that have signed an agreement with the 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, the ITPGRFA's standard material transfer agreement to plant genetic resources for food and agriculture not listed in Annex I to the ITPGRFA, which means that the conditions of the Multilateral System, ostensibly, also apply to those crops.

At its fifth session in 2013, the Governing Body of the 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 DSI should be addressed under the Treaty and whether to expand Annex I of the Treaty to cover food and forage crops or perhaps even all PGRFA. 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 if and how this work may continue.

It is worth noting in support of this process, in 2017 the ITPGRFA Secretary commissioned a 'Scoping Report Potential implications of new synthetic biology and genomic research trajectories on the International Treaty for Plant Genetic Resources for Food and Agriculture' (Welch et al., 2017) to explore how current technologies and practices related to the exchange and use of genetic information are relevant for the ITPGRFA.

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 CBD and its Nagoya Protocol (as noted in section 8.1.5 and 8.4.1) is mirrored under the 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 the ITPGRFA, 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 (Moore & Tymowski, 2005).

(b) *The United Nations Convention on the Law of the Sea (UNCLOS) proposed agreement for marine biodiversity beyond national jurisdiction (BBNJ).*

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 157 Parties.

The specific question of international governance of marine genetic resources in areas beyond national jurisdiction (ABNJ) was not addressed in the 1982 agreement. As a result, there is no comprehensive global framework for the conservation and sustainable use of marine areas beyond national jurisdiction to halt and prevent further degradation from human activities.

Since 2004, the international community has discussed the need to close the existing ABNJ governance gap and ensure the conservation and sustainable use of biodiversity in these areas. In its resolution 72/249 of 24 December 2017[91], the General Assembly of the United Nations decided to convene an Intergovernmental Conference, under the auspices of the United Nations, to consider the text of an international legally binding instrument under UNCLOS on the conservation and sustainable use of marine biological diversity of ABNJ.

An Intergovernmental Conference on an international legally binding instrument was convened from 4 to 17 September 2018 with second and third sessions taking place in 2019. A Fourth session is scheduled to

[91] https://undocs.org/en/a/res/72/249

take place 16-17 August 2021 to consider text for the instrument[92]. The negotiations are focusing on a global framework for marine protected areas in ABNJ, ensure states assess impacts of potentially harmful activities, and facilitate inclusive scientific research that enables the equitable sharing of benefits from marine genetic resources. The intersessional program[93] of work of the Intergovernmental Conference includes the following elements which are relevant to the Convention on Biological Diversity, particularly its third objective concerning equitable benefit-sharing as well as the objectives of its Nagoya Protocol:

- modalities for access and benefit sharing
- the role of traditional knowledge
- modalities for capacity-building and the transfer of marine technology
- relationship with relevant legal instruments and frameworks and relevant global, regional, subregional and sectoral bodies
- international cooperation and coordination in relation to measures such as area-based management tools, including marine protected areas.

(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'[94].

WIPO's Background Brief[95] 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 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 (GRs) 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: (i) traditional knowledge, in the narrow sense, refers to practical knowledge, including know-how, practices, skills, and innovations; (ii) 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; (iii) GRs, where IGC aims to complement the frameworks for access and benefit-sharing of GRs utilisation. In a broad sense, traditional knowledge describes intellectual and intangible cultural heritage, practices, and knowledge systems of IPLCs, including the three thematic areas.

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[92] https://www.un.org/bbnj/

[93] https://www.un.org/bbnj/sites/www.un.org.bbnj/files/bbnj_intersessional_programmeofwork_210114.pdf

[94] WIPO, *Intellectual Property and Traditional Cultural Expressions/Folklore*, https://www.wipo.int/edocs/pubdocs/en/tk/913/wipo_pub_913.pdf

[95] https://www.wipo.int/publications/en/series/index.jsp?id=144

The WIPO General Assembly, which took place from September 30 to October 9, 2019 renewed the 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 (GRs), traditional knowledge and traditional cultural expressions[96]. 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. This has 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 next meeting of the Committee, IGC 41, which was planned to take place from 16-20 March 2020 is yet to be rescheduled.

9.4. Other International laws, processes and initiatives which are relevant to the objectives of the CBD.

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.

According to its Article 7 (objective), 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 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. For components, organisms and products resulting from synthetic biology techniques, patents and protection of plant varieties are most relevant, but 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 TRIPS (apart from general non-discrimination principles) until 1 July 2021, a deadline that has been extended twice and may be extended again[97].

Overview of main provisions:

- 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 (UNCTAD-ICTSD 2004).
    - 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

[96] https://www.wipo.int/export/sites/www/tk/en/igc/pdf/igc_mandate_2020-2021.pdf

[97] At the time of writing the continued exemption of least-developed countries (LDCs) from TRIPS obligations issue was under consideration as per the proposal contained in document IP/C/W/668, most recently considered at a meeting of the Council for Trade-Related Aspects of Intellectual Property Rights (TRIPS) on 10-11 March 2021.

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).

- It has been argued that many components, organisms, and products resulting from synthetic biology techniques fulfil these criteria. In particular, 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 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 micro-organisms, 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 micro-organisms, 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 micro-organisms which include 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 TRIPS.
- 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[98]. UPOV has 77 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.

- <u>Overview of main provisions.</u>

  - 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.

The Explanatory Notes on the Definition of Variety under the 1991 Act of the UPOV Convention (document UPOV/EXN/VAR/1) states as follows:

> *"4. The definition of "variety" under the 1991 Act of the UPOV Convention starts by stating that it is "a plant grouping within a single botanical taxon of the lowest known rank, …" thereby confirming that a variety may not, for example, consist of plants of more than one species.*
>
> *"5. The definition that a variety means a "plant grouping" clarifies that the following, for example, do not correspond to the definition of a variety:*

[98] Unless otherwise stated, reference to the UPOV Convention in the following refers to the 1991 Act of the UPOV Convention.

- *a single plant; (however, an existing variety may be represented by a single plant or part(s) of a plant, provided that such a plant or part(s) of the plant could be used to propagate the variety)*
- *a trait (e.g. disease resistance, flower color)*
- *a chemical or other substance (e.g. oil, DNA)*
- *a plant breeding technology (e.g. tissue culture).”*

- Breeder’s rights

In order to be eligible for protection, a plant variety must meet the following requirements (Article 5 UPOV Convention):

“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 UPOV Convention). […]” 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 (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, requires the authorization of the breeder (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 rights

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 harmonise 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 recognises 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 organisations, but so far there has never been a proposal to recognise 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 organisations. 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 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 organisations 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.

As discussed in section 4 on potential impacts, of components, organisms and products resulting from synthetic biology may be intentionally or unintentionally released to the environment, leading to biosafety 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 summarised in subsection “Sanitary or phytosanitary measures” above.

A WTO dispute between the USA and the European Communities (EC) concerning ‘Measures affecting the Approval and Marketing of Biotech Products’ (EC-Biotech) case concerns 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 2015 technical series. 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.

- 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 *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 regards 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 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). 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 organisation 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 minimise 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[99] 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 2 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 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 products moving in international trade, it also covers research materials; biological control organisms; germplasm banks; containment facilities and anything else 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 1, paragraph 3 IPCC).

ISPM No. 11 ‘Pest risk analysis for quarantine pests’ addresses LMOs. It notes the types of LMOs that a national plant protection organisation (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;

[99] Available at: www.ippc.int/core-activities/standards-setting/ispms#block-agenda-items-list.

- pests modified to alter their pathogenic characteristic and thereby make them useful for biological control (see ISPM 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 of ISPM No. 11 provides guidance for 'Determining the potential for a living modified organism to be a pest'. It clarifies further, for the case of living modified organisms that for phytosanitary risks related to gene flow, the living modified organism is acting more as a potential vector or pathway for introduction of a genetic construct of phytosanitary concern rather than as a pest in and of itself. Therefore, the term "pest" should be understood to include the potential of a living modified organism to act as a vector or pathway for introduction of a gene presenting a potential phytosanitary risk. Annex 3 of ISPM No. 11 contains a list of potential phytosanitary risks from living modified organisms. All these risks may apply, to varying degrees, to components, organisms and products resulting from synthetic biology.

Other ISPMs which have been identified as relevant to living modified organisms (Secretariat of the Convention on Biological Diversity, 2012), and therefore may in some cases be relevant to components, organisms and products resulting from synthetic biology, include:

- ISPM No. 12: Guidelines for phytosanitary certificates (2001)
- ISPM No. 7: Export certification systems (1997)
- ISPM No. 3: Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms (2005)
- ISPM No. 20: Guidelines for a phytosanitary import regulatory system (2004)
- ISPM No. 23: Guidelines for inspection (2005).

(c) *World Organization 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 organisation has been expanded to become the improvement of animal health world-wide.

The OIE standards, recognised by the SPS Agreement as the international standards for animal health including zoonosis, 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.

- <u>Sanitary measures.</u>

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 micro-organisms, 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 (FAO) and the World Health Organization (WHO) that was set up to establish international standards on foods[100].

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 organisations 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 harmonising 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)).

Documents relevant to components, organisms and products resulting from synthetic biology include, for example[101]:

- 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 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 apply 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. For an analysis see therefore sections 8.2 and 9.4.2(e) above.

F. 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 to the l governance of synthetic biology. Potential challenges and opportunities for synergies and cooperation associated to this scenario are also discussed.

It is also worth noting that when doing an analysis of potential gaps and overlaps associated with the governance of synthetic biology, it is recognised that the wide range of applications of synthetic biology,

[100] For an introduction to the Codex Alimentarius see http://www.codexalimentarius.org/about-codex/en/.

[101] These documents are available online at www.codexalimentarius.org/standards/list-of-standards/.

as well as its cross-cutting nature, makes it difficult to draw a clear line of action or intervention for some international initiatives, and as such, "overlaps" does not necessarily mean duplication, but instead could be related to the fact that a particular aspect of synthetic biology could be discussed or considered under more than one initiative, but 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.

Although synthetic biology is often referred to as a single discipline, the lack of consensus on a clear definition and the numerous areas of synthetic biology research and the wide-array of applications and potential impacts can create challenges in assessing potential gaps and overlaps associated with the work done by the Convention, and its Protocols with other international laws, processes and initiatives which could have potential implications for the international governance of synthetic biology. In this sense, what is immediately apparent from the international-level mapping described in Sections 8 and 9 is that no specific governance or process of rule-making on an international scale exists to 'regulate' synthetic biology. The extensive regulatory instruments and mechanisms apply to all or some forms of synthetic biology depending on the application.

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 the term synthetic biology became widely used and therefore, they were not developed with the necessary scope and scale that some of the potential impacts of synthetic biology may present.

However, within this fragmented landscape, it is evident that 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 on 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 COP decision-making explicitly addressing synthetic biology on issues such as its relevance towards the objectives of the Convention and its Protocols, biosafety risks associated with LMOs, implications concerning access and benefit sharing and the participation of IPLCs amongst others.

In parallel, other instruments and organisations, 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 organisations, 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 contextualised 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, as well as opportunities to consider not only risk assessment and mitigation approaches in a tailored manner, but also relevant governance implications under international laws, processes and initiatives.

The 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 will also be discussed.

10.1. 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 offers 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 transboundary movement of LMOs derived from synthetic biology. 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.

Additionally, its 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 have reaffirmed the importance of participatory decision-making by IPLCs and their adequate consultation in the context of biosafety.

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 to the use of synthetic biology techniques 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, 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 of the substantial body of work to date under the Convention and its Protocols.

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 UN 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 COP to the Convention have explicitly recognised the importance of FPIC of IPLCs in the context of the environmental release of engineered gene drive organisms, however, as noted in Section 10.6., challenges remain in the translation of FPIC principles into effective and standardised protocols for community consultations and participatory-decision-making.

In instances in which synthetic biology organisms or applications were outside of the scope of the Convention and its Protocols, 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, may provide some guidance relevant to addressing potential negative impacts resulting from the application of synthetic biology techniques; however, these would still form an incomplete basis to address all potential positive and potential negative impacts derived from the range of uncertainties from their application especially in the absence of specific guidance. Additionally, self-regulation by the synthetic biology community may also play an important role in mitigating the risk of harm, whether to the environment and biodiversity, or 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 synthetic biology 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.

Gaps could occur where components and products resulting from synthetic biology techniques do not fall within the scope of a treaty regime.

(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 which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity.

There are other international organisations (e.g. WHO) with mandates that are directly related to human health issues. This somehow implies that there could be potential interactions amongst various organisations in relation to applications of synthetic biology which have implications for human health. In a containment context, for instance, the Laboratory Safety Manual of the WHO provides guidance regarding biosafety in the context of clinical and public health, and also in contexts which 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 ease with which it can be manufactured using synthetic biology techniques in the absence of appropriate safeguards could be seen as complementary to the Cartagena Protocol on Biosafety. In a public health context concerning for instance the environmental releases of synthetic biology health-oriented applications (e.g. mosquitoes with engineered gene drives), potential interactions could arise as different organisations (e.g. CBD 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. These have implications for commerce and trade.

10.2. Conservation.

Some considerations under the Convention and its Protocols which are relevant to the conservation of biodiversity haves focused on its 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 for instance under the IUCN given its primary focus on nature conservation and sustainable development. Unlike the Convention and its Protocols, the IUCN is not established by contracting Parties under an international treaty, however, given its comprehensive programme on synthetic biology initiated in 2016, including its technical assessment on synthetic biology undertaken in 2019 and in-progress principles proposed to be adopted on synthetic biology conservation, there are strong synergies with the activities under the Convention and its Protocols focusing on synthetic biology governance.

10.3. 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 underway to resolve a divergence of views amongst Parties regarding the benefit-sharing obligations arising from the use of digital sequence information on genetic resources may have implications for synthetic biology organisms and applications developed using 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. This includes in a non-exhaustive manner, the

International Treaty on Plant Genetic Resources for Food and Agriculture, the Pandemic Influenza Preparedness (PIP) Framework of WHO and the negotiations towards an agreement for marine biodiversity beyond national jurisdictions, all of which are likely to consider the outcomes of the process under the Convention and its Nagoya Protocol to inform their own deliberations on this issue.

10.4. 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 utilisation of genetic resources in their development, it can be seen to have implications for commerce and trade. A number of other international agreements regimes and initiatives exist which, in general, may also have implications associated with commerce and also trade. CITES is particularly relevant in the context of synthetic biology especially 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 or work addressing synthetic biology however, given the extent to which they underpin 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, the IPCC, the OIE standards, as well as related instruments such as the Codex Alimentarius which establishes standards on food, are likely to have significant implications for the governance of synthetic biology.

An additional challenge specifically relates to the identification and detection elements for the organisms of synthetic biology applications. Many potential synthetic biology organisms may not be easily or feasibly detectable 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 (Section 6.2.5.).

10.5. 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, there is a perception that synthetic biology is still not yet delivering on its promise (El Karoui et al., 2019).

There is an apparent knowledge gap in our understanding in how nature works. This makes it difficult to apply the DBTL 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 knowledge gap is also related to challenges associated to the risk assessment of 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 characterise 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 "standardisation" especially around DNA design.

10.6. International governance: social, economic and cultural concerns.

Social, economic, and cultural considerations are as equally important as the consideration of potential impacts on biodiversity, conservation and sustainable use during decision-making and governance of synthetic biology applications. However, as relatively few synthetic biology applications have reached commercialisation/wide release, relatively little "real world" data has been collected, including those that may reflect benefits. Thus, the range of potential impacts on the conservation and sustainable use of biodiversity remains largely hypothetical/speculative and is informed mostly by previous experience with classical genetic engineering and associated concerns. Further, the evaluation and/or quantification of any benefits has been somewhat absent 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 socio-economic and cultural considerations will be very context-specific.

Inclusive decision-making and community engagement are needed to steer responsible research and use of synthetic biology applications. One approach that has already been championed concerns Free, Prior Informed Consent (FPIC). Given the prominence of FPIC in the preamble and provisions of the Convention, as well as in the recommendations and decisions of its decision-making bodies, Parties must 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. The concept of FPIC has grown steadily in prominence in the context of conservation and land development decisions impacting IPLCs, and society at large. FPIC is an evolving concept and focuses on mechanisms to increase the participation of society in all stages of technology development and application. Further, FFIC 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 IPLCs should be able to conduct their own independent and collective discussions and decision-making. Decision XIII/18 from the COP[102], for instance, invites international funding institutions and other actors to consider, consistent with their mandates, providing financial and technical assistance to amongst others, IPLCs to develop, as appropriate, community protocols or processes for "prior and informed consent", "free, prior and informed consent" or "approval and involvement", depending upon national circumstances. 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 however continues to prove challenging. Therefore, although IPLCs participate in several processes of the CBD, their involvement is considered insufficient compared to the diversity of IPLCs worldwide, due to several underlying factors, including: limited government support and recognition; lack of economic resources; language barriers, and; gender issues. There is a need for the translation of information for IPLCs in their languages, and it is imperative to give IPLC representatives adequate time and space to express their needs and concerns, so that they are effectively represented in decision-making processes during CBD COPs and other such meetings (Fajardo et al., 2021).

When it comes to enabling or improving community engagement in the development and deployment of synthetic biology research, challenges are likely to be faced on several fronts, but primary amongst them is actually one of the first steps and concerns developing relationships with communities to the extent that their involvement is encouraged and maintained. This is especially key when working with non-traditional communities. Assisting the process would be the creation of a community advisory board to be involved in the development of research project and application in a manner that help 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

[102] https://www.cbd.int/decisions/cop/13/18

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, 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 conceptualised in order for meaningful public participation and FPIC. An early example of such material is the common glossary co-developed between local communities, linguists and researchers focussing 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 policy-makers. Examples include the Scientific Citizenship Initiative[103] 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[104] at New York University (USA) has initiated a holistic approach to artificial-intelligence research that incorporates inclusion, bias and justice. And Editing Nature[105] provides platforms that integrate scientific knowledge with diverse cultural world views to foster the responsible development of environmental genetic technologies.

As we think about moving synthetic biology into the future, the challenge is integrating the scientific freedom that allows research and product development to move ahead while acting responsibly and in a manner that embraces ethical, legal, and larger societal values.

10.7. Implementation of regulatory frameworks.

The greatest focus of regulatory frameworks which have implications for the governance of the synthetic biology and which are relevant to the objectives 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 complimentary disciplines 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 of 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 potential applications of synthetic biology is far exceeding the speed at which national and international governance frameworks 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. As in the case of challenges arising from the differences between a product-based and a process-based approach to regulation for classical genetic engineering, it is to be expected that similar if not greater challenges will continue to be faced for those organisms resulting from synthetic biology.

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[103] https://sci.hms.harvard.edu/

[104] https://ainowinstitute.org/

[105] https://www.editingnature.org/

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 synthetic biology applications approach commercial deployment and potential environmental release, engineered gene drives provide a useful lens through which to evaluate overlaps and potential gaps in the governance of synthetic biology.

The CBD and its Cartagena Protocol on Biosafety are framed primarily by the objective of conserving and protecting biodiversity, whilst also taking into account human health whereas the primary goal of the WHO is the development of effective and safe health interventions. The release of a disease vector LMO with an engineered gene drive will require assessment and approval by national authorities. In assessing such an application, a field evaluation will likely be suggested as per a step-wise 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 a positive impact for disease control. Such diverging orientations could pose practical challenges in the design of field evaluations of engineered gene drive organisms, especially when aiming to minimise risk while demonstrating positive health impacts. It shows that issues of interaction and coordination are potential shortcomings under a fragmented international regime. Such shortcomings have the potential to be further perpetuated and exacerbated by the absence of integrated guidance provided under each regime or implementation under national law. The WHO, for example, has published guidance of a framework to establish best practice for research into genetically modified mosquitoes which includes recommendations on biosafety, ethics, regulation, and efficacy, in addition to gene drive-specific recommendations. On the other hand, under the CBD and its Protocols, discussions are ongoing regarding potential additional risk assessment guidance for organisms 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).

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 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 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.

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, amongst other factors.

11. Conclusions

Since the publication of the previous technical series on synthetic biology in 2015, synthetic biology has continued to advance exponentially. With a broader array of applications nearing commercial release and new promises to solve global challenges, the discipline has garnered wider awareness and attracted much attention. This has also brought heightened awareness 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 become available, there is a growing pressure towards achieving clarity.

A comparison between the synthetic biology applications listed in the Technical Series No. 82 and those in the present document indicates that the number that are commercially available or in advanced development stages has shown significant growth, which goes in line with the increasing activity and interest in synthetic biology. As was the case in 2015, applications for contained use still represent the majority of 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, engineered bacteria fertilisers and self-limiting insects. Additional products intended for environmental release are in advanced stages of development, such as genome edited animals and organisms containing engineered gene drives to control vector-borne diseases. Also, in the case of research and development, time has shown that the relationship between early research and development and the commercialisation of products is not linear, and not every application that is under research and development will eventually reach an advanced stage or be commercialised.

Despite these recent developments, there is a continuing need to acquire further 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 (i.e those discussed in the present document) were developed before the term synthetic biology became widely used, and therefore may not have 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 and discussions under 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, and although existing 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, the inability to potentially detect and identify the applications of synthetic biology adds complexity and may strain the abilities of developing nations whose regulatory frameworks may not be (fully) developed.

A common feature of articles identifying gaps or deficiencies in the governance of synthetic biology focus on the operation of international regimes as silos and the need to firstly better integrate/coordinate governance of synthetic biology and secondly, 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. In a research and innovation context this holistic approach is manifest in calls for so called “Responsible Research and Innovation” to assess societal implications of emerging technologies to better align process and 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 responsibility for expensive clean-up and/or insurance efforts (Trump et al., 2020) and 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 also evident in 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). Such novel mechanisms are suggested as a means of better equipping the international governance framework as 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 IPLC under the Convention and has recently been underscored in COP decision 14/19.

There is evidence of scientific research addressing community engagement in field trials, concerning for instance engineered gene drive organisms (i.e for malaria control). 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 had considered the views, preferences and needs of the community in designing their approach. This engagement process has prompted the researchers to use only white-footed mouse DNA, meaning the current project will not involve gene drives. Through this process, community members were continually engaged and given the opportunity to share their suggestions and concerns; a process that has already identified potential ecological consequences unanticipated by the research team that will likely affect implementation (Buchthal et al., 2019).

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 standardised 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 in this regard appears a logical next step and given the continuously evolving nature of the principles underpinning FPIC, such guidance may benefit form closer evaluation of approaches involving Responsible Research and Innovation, Governance Coordinating Committees and principles of transparency, accountability, integrity, and capacity noted above. 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 organisations with a substantive mandate and programmes on synthetic biology to address relevant issues that require a consolidated and coordinated approach.

By applying novel genetic techniques, synthetic biology offers the opportunity to modify organisms to address unprecedented environmental challenges. Despite its potential benefits, the intentional or accidental release of products from synthetic biology into the environment could have significant 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. In addition, considering the fast pace of development of synthetic biology, and the challenge for regulatory regimes to cope up with potential new applications, an early screening of what is under research and development and commercialisation perspectives of those products would be critical in providing timely information for countries and organisations 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 laws, processes and initiatives analysed appear ill-equipped to address several of these dimensions.

The overlaps and gaps identified in this update suggest that opportunities exist for increased coordination amongst the Convention and its Protocols, and with other relevant international treaties, processes and initiatives converging on the governance of synthetic biology. Such multilateral coordination could minimise 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 COP decision VII/26 (paragraphs 1 and 2), its

implementation of a roadmap to optimise synergies, as well as the creation of a group to steer the process in the lead up to the adoption of the Post-2020 Global Biodiversity Framework at COP 15 could be instructive in this regard. Similarly, other models of multilateral initiatives to facilitate international governance on cross-cutting issues relevant to the objectives of the Convention could also be considered.

The cross-cutting nature of synthetic biology, added to its broadness as a discipline, 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 a number of regulatory mechanisms. While some instruments are sufficiently broad to address some of the current issues related to synthetic biology, gaps still exist relating to the practical implementation of these instruments. Furthermore, 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.

Finally, as synthetic biology will continue to grow in relevance and importance due to the opportunities that it offers towards solving global challenges, it is imperative that resources are available concurrently for research and development, and 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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