Submission on information and supporting documentation relevant to the trends in new technological developments in synthetic biology and any additional trend that should be considered.

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Abbreviations

AHTEG	Ad Hoc Technical Expert Group
CBD/Convention	Convention on Biological Diversity
IPR	Intellectual property rights
GMO	Genetically modified organism
LMO	Living (genetically) modified organism
Protocol	Cartagena Protocol on Biosafety
SynBio	Synthetic biology

This submission will emphasise items (b) Increased development of technologies that genetically modify organisms directly in the field, (c) A shift to the development of synthetic biology for environmental, conservation, agricultural and health uses, (d) Increasing sophistication of methods, including, for example, new genome editing techniques, more complex metabolic engineering, the recoding of genomes, and the use of artificial intelligence/machine learning for the redesign of biological systems, (e) The use of transient modification of organisms, including, for example, through the use of synthetic double-stranded RNA molecules, nano-particles and genetically modified viruses, and (f) Ability to produce new synthetic biomolecules using non-canonical nucleotides and amino acids referred to in paragraph 6 of decision 15/31, which correspond to the trends identified in the report of the 2019 Ad Hoc Technical Expert Group on Synthetic Biology at paragraph 5. In the first two sections of our submission the items are not individualised because the various techniques of synthetic biology can be used in combination or may involve risk issues that transcend the individual items. *We contend that an important additional trend is where these techniques combine leading to heretofore unprecedented scales at which possible benefit but also potential harm can be created.*

Our submission is guided by Article 1 of the Convention on Biological Diversity. In this Article, three objectives are articulated. They 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 utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding." In the final section of this submission a proposed alignment between the objectives is provided to suggest and prioritise the emerging issues that require specific further development and better knowledge sharing.

I. An emerging trend is to use techniques in a series and at scale.

Technological developments identified by the SynBio AHTEG are those that increase the efficiency of achieving a particular change at the DNA level (Heinemann 2022; Touzdjian Pinheiro Kohlrausch Távora et al. 2022). The increase in efficiency makes it possible to make more intended changes in less time or in more individuals in a coordinated fashion (e.g., as might be done in outdoor use), but to also build on previous changes in ways that previously were not possible except in a very few organisms, such as *Saccharomyces cerevisiae* (yeast) or *Escherichia coli* (bacteria).

The very appeal of the underlying techniques used in synthetic biology is their ease of use in so many species, rate at which intended outcomes can be achieved, and the range of changes that can be made in the same cell at the same time. It is to be expected that these industrial qualities will be exploited.

Various techniques used in synthetic biology may be combined for the production of an organism, or may be used in series over a large time period time on organisms that were previously modified using other synthetic biology techniques, building a diverse succession of products. All these properties create scale changes.

Scale changes amplify risk. Risk is the (probability of harm) x (severity of harm). Scale amplifies risk by increasing the probability of harm through the number or types of hazards that may be created, and/or the severity of harm by changing the number of types of exposures that become possible (Heinemann et al. 2023).

For example, the technology used to concentrate uranium atoms that are, in most natural settings, nearly harmless, into a nuclear warhead is a scale change. The technology behind the

warhead makes it possible to alter the hazard of radiation and energy resulting from the spontaneous decay of atoms into a chain reaction of atomic decay at a time and place where many people live. The technology simultaneously changes both the probability and severity of harm by changing potential exposures and magnitude of released energy.

The fundamentally new capacity in the genetic engineering possible for plants and animals (and many other kinds of organisms) inherent in the techniques of synthetic biology also creates new risks (as will be elaborated below). Those risks are compounded should individual techniques that are used in synthetic biology be released from regulation and obligations to monitor and report.

Each technique every time it is used can result in novel legacy risk issues (Chu and Agapito-Tenfen 2022). These may fail to be taken into account depending on how or if different synthetic biology techniques are regulated or not regulated. If regulated, regulatory authorities must be competent to evaluate risk and empowered by harmonious approaches to continued monitoring and assessment as can be provided under the Convention.

It is important to consider combinatorial uses of techniques, particularly those intended to "reverse" the effects of techniques earlier in the process, or to result in "transient modifications". Each of these creates its own unknowns (Agapito-Tenfen et al. 2018). We will discuss two examples of the above. One (null segregants) involves first making an LMO and the other (transient modifications) involves no prior "In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles" (CBD 2003).

i. Null segregants

Synthetic biology techniques[1] could be used to remove material added to an organism, with the intention of attempting to convert an LMO back into an unregulated organism or out of scope of the Protocol. Such procedures can produce null (or negative) segregants (ASSAf 2016). A null segregant is a product of a prior use of modern biotechnology/SynBio followed by a second use of modern biotechnology/SynBio technique(s).

Setting aside the philosophical debate that a genome, much less an organism, can be in every relevant sense restored to a form that was never altered by human intervention, no technique has yet been invented that has no secondary or unintended effects. The sub-atomic scale at which biochemical reactions occur make them subject to variances that cannot be completely controlled by biotechnologists any more than a physicist can simultaneously control both the location and speed of an electron[2].

Moreover, the reagents used have a lifetime that is not controlled at a time scale that is relevant to the rate of a reaction. The relevance of this is that the average time required for a technique to make the intended change in a sufficiently large number of genomes (necessary for the biotechnologist to have confidence the intended genetic change has been made) is greater than the time needed for a number of other unintended changes to be made in every cell (Heinemann et al. 2023).

The null segregant is thus the end of at least two series of interventions on the gene level, not an organism that has never been modified by virtue of the last series of

[1] "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" (CBD 2022).
[2] We refer here to the Heisenberg Uncertainty Principle.

interventions. For regulatory purposes, the question becomes whether the null segregant can be *predetermined* to have no characteristics that would make a scientific risk assessment worthwhile, or which would have no socioeconomic, cultural, or ethical implications.

We submit that each technique used in the series of interventions warrants the use of a risk assessment because the intended outcome of each technique is not the actual outcome until it is proven to be (Buchholzer and Frommer 2023). No product enjoys a predetermined "as safe as" standard. There are too many examples of erroneous claims from various manufacturers of genetically modified organisms intended for commercial use to justify confidence that the actual outcome is the intended or claimed outcome (see for example: Rang et al. 2005; Solomon 2020; Windels et al. 2001). Only proper guidance provided to regulatory officials to verify such claims can assure that they are accurate.

ii. Transient modifications

There is no universal definition of a transient modification. It is often used to mean one of two different things. The first is that any nucleic acid reagents used have a limited persistence in the cell/organism. An example is to insert into a cell the mRNA of a site-directed (genome editing) nuclease such as Cas9 along with an RNA oligonucleotide to guide the nuclease activity. The second is that the modification to the organism's phenotype may not persist. An example is to induce an RNAi effect (i.e. post-transcriptional gene silencing and/or RNA-directed DNA methylation) in a plant or animal without inserting new DNA into the genome.

Transient is a term that lacks the precision needed to regulate risk from the use of synthetic biology. Does it mean a modification that cannot persist beyond reproduction? If so, would a modified tree that could live 1000 years but not reproduce be only transiently modified? What if the modification were transmitted by grafting but not sexual reproduction (Palauqui et al. 1997; Whangbo and Hunter 2008)? Is it transient if it persists for only one additional generation, 20 generations, 80? Exposure to exogenous dsRNA molecules can result in phenotypes that persist for almost one hundred generations, possibly indefinitely in some species (Houri-Zeevi and Rechavi 2017; Vastenhouw et al. 2006).

There is no scientific principle upon which to predetermine how long a phenotype can exist before it causes a harm (Heinemann 2019). Only a risk assessment can attempt to do this.

a. Transient reagents

A common technique in plant biotechnology is to insert into a cell either a proteinaceous sited-directed nuclease (and guide oligonucleotide if required), or the mRNA to be translated into a nuclease, rather than insert a transgene for the ongoing expression of these things.

In such cases the reagents used to make modifications are not taken into the "genome", generally meaning the DNA content of the cell. The reagents effect changes in the DNA molecules of the genome and then are intended to disappear over time or dilute as the cells divide. How long this takes is dependent upon how many such molecules enter a cell and how stable the molecules are. Modified nucleotides may be used to significantly enhance and extend their biological activities (Nance and Meier 2021; siRNAmod 2018).

Loss of the mutagenizing agent (transient reagents) in the manner described above has been likened to the use of chemical or radiation mutagens. Those mutagens have been used since the 1920s to increase the rate of genetic change in exposed organisms, such as crop plants. In both cases the modifications made by transient reagents are not transient. This observation was invoked unsuccessfully before the High Court in New Zealand to equate chemical or radiation mutagenesis with the use of SynBio transient reagents based on a superficial analysis of arbitrary biochemical similarities between these processes. Neither the New Zealand nor the European courts found such arguments compelling (Heinemann et al. 2023).

The two different mutagenesis processes are alike in being technologies that increase the scale of production of genetically modified organisms. That is why many countries define the use of chemical/radiation mutagenesis as a process for creating GMOs. The key distinguishing difference between chemical/radiation mutagenesis and techniques directed at specific nucleotide sequences is not the biochemistry behind the reactions, but instead that the former is already regulated through different legal instruments and the latter may only be regulated effectively through GMO provisions (Heinemann et al. 2021). Therefore the use of chemical/radiation mutagenesis is exempted from the additional GMO regulations brought in for techniques such as result in transgenic or genome edited organisms (Heinemann et al. 2023).

Unless the techniques of SynBio are taken under the umbrella of GMO regulations, they will be unregulated altogether. This is a novel risk scenario because no other technology that concentrates mutagens is entirely unregulated.

b. Transient phenotypes

Transient phenotypes may be descriptive of a wide range of applications. As described above, an elevated mutation rate in an organism that contains transient mutagenic agents is a transient phenotype even though the final intended mutation is not. Beyond this, use of techniques that result in death, as may be the intend of a pesticide, could be seen as transient because the organism never will have offspring with the modified gene or trait. Techniques that cause a limited duration change in growth rate, flowering time, age of fertility, colour, rate of ripening/senescence, or virus resistance could also be transient in some manifestations of the art.

Both dsRNA and modifications of genome editing nucleases (Zhang et al. 2021) are used to alter gene expression and are imagined for the activities listed above (Sirinathsinghji 2019), either by design or through unanticipated secondary activities of the reagents (Sharma et al. 2022).

The duration of the phenotype provides no predictive power for a *predetermination* of risk. The longevity of a phenotype may not relate to the longevity of the activity of biological or chemical reagents used to cause the phenotype. Modified nucleotides of nucleic acids, or constructed higher level structures in nucleic acids (Nance and Meier 2021), or nanomaterials, alter their stability (Heinemann 2019; Heinemann and Walker 2019). They may persist in a biological or environmental state for much longer than expected (Parker et al. 2019). Some reagents may move through food chains (FIFRA 2014; Garbian et al. 2012; Murphy et al. 2016; Whangbo and Hunter 2008; Zhang et al. 2012).

> A phenotype may be caused by epigenetic changes that only under certain conditions appear to be transient. By its very nature, epigenetic inheritance can be unstable. That does not mean that it always is. Epigenetic changes resulting from gene silencing, as mentioned above, have been monitored to 80 generations in multicellular eukaryotes (Houri-Ze'evi et al. 2016; Houri-Zeevi and Rechavi 2017), and 200 generations in bacteria (Novick and Weiner 1957).
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> Therefore, the intended receiving environment may not be the last environment to hold the reagents that cause a phenotype intended to be transient. The regulatory community requires guidance materials for conducting fit-for-purpose risk assessments.

All three objectives of the Convention are put at risk by this trend. Previously, the issue of scale was the size of the population of LMOs (e.g. GM maize in a centre of origin). The issue is changing into the rate at which genetic engineering can be done and the environmental area (see Section II) at which it can be done. Both rate and area are changes that alter the magnitude of properties of risk. Potential hazards to biodiversity can be created faster and over larger areas, threatening sustainable use of its components. Because the potential hazards are products of modern biotechnology techniques, they have available to them the power of distribution and concentration that comes with uniquely powerful intellectual property rights protections. That socioeconomic effect may further erode both sustainable use of components and the fair and equitable sharing of benefits.

II. Synthetic biology is co-developing with new chemistries for genetic engineering outside of containment

Perhaps the most significant difference in the options for genetic engineering available in the 20th Century to those becoming widespread in the 21st Century is not the new reagents (sometimes confused with being new techniques) of genome editing and gene silencing. Instead it is the ability to cause intended genetic changes at landscape scales at such high efficiency that genetic engineering could be done outside of laboratories.

The capacity to conduct large-scale genetic engineering or gene silencing for synthetic biology is referred to in the scientific and patent literature by various names, including environmental genetic engineering or environmental RNAi (when it is only about the use of dsRNA) or variations of these (Heinemann 2019; Li et al. 2021; Whangbo and Hunter 2008). It is supported by co-technologies that also have no consensus terminology and range from penetration to topical and transformation technologies (Heinemann and Walker 2019; Sirinathsinghji 2019).

The chemical co-formulants that are or may be used to ensure efficient translocation of a protein or nucleic acid molecule into cells are developing at pace. Because these are considered formulations that support active ingredients, regulators are being tempted to assess their risk in a hazardous substances rather than GMO framework. Such frameworks are inadequate to protect the objectives of the Convention (Li et al. 2021; Zeng et al. 2022).

From the view of hazardous substances, the products may be prematurely predetermined to be safe. This is because each of the individual components may be considered chemically safe. For example, DNA/RNA are safe chemicals; water is a safe chemical; detergents can be safe chemicals. The functionality of the mixture may not be tested provided that the active ingredient and individual formulants is predetermined to be safe (Heinemann 2019). Often, even chemical components are not tested for safety and when they are, it is only for risk to human health (Krimsky 2017; Landrigan et al. 2018). Where environmental endpoints are included, they may be specific to certain species but not the functional range of species in the

intended receiving environment. This framework falls well short of the objectives of the Convention.

> A HIGH PROPORTION OF THE 140 000 CHEMICALS AND PESTICIDES IN COMMERCE HAVE NEVER BEEN ADEQUATELY TESTED FOR SAFETY OR TOXICITY. INFORMATION ON POTENTIAL TOXICITY IS PUBLICLY AVAILABLE FOR ONLY ABOUT HALF OF THE COMMERCIAL CHEMICALS WITH HIGH PRODUCTION VOLUME THAT ARE IN WIDEST USE, AND INFORMATION ON DEVELOPMENTAL OR REPRODUCTIVE TOXICITY IS AVAILABLE FOR FEWER THAN 20% OF THESE WIDELY USED CHEMICALS. (Landrigan et al. 2018)

While novel chemistries such as nanomaterials are being promoted for environmental use (e.g. Mitter et al. 2017), they are not always needed. The patent literature already describes the use of physical rather than chemical "co-formulants". Active biological molecules in only water or saline may be coupled with epidermal abrading using lasers or high pressure washes (Heinemann 2019; Heinemann and Walker 2019).

The chemical or physical vectors of active ingredients of techniques used in synthetic biology allow for scale changes from the *mechanisation* of environmental genetic engineering. Again referring to the patent literature that we have described elsewhere (Heinemann 2019; Heinemann and Walker 2019), airplanes and drones that canvass large agricultural fields are being tested to cause mass outdoor use of modern biotechnology in annual crops which, because harvested each season, may be argued to have only transient phenotypes. Likewise, sprays are envisioned for use in food stores as a means to make the produce appear attractive for longer.

While the intended change in the intended organism may be assessed and approved by a regulator based on separate testing in laboratories, the use of the agents in the out of doors negates the value of the tests. This is because any strategy for augmenting the transfer of the reagents used in the techniques of synthetic biology into the cells of organisms, are incapable of restricting themselves to the intended target organism.

As we've said before, biodiversity in potentially exposed environments is both high and largely undescribed (USDA ; Womack et al. 2010) making it both difficult to limit exposures to intended species and predict the effects of all exposures. Airborne microorganisms including eukaryotes can number in the hundreds of thousands per m^3 and "can be as diverse as those in terrestrial environments, including soils" (Womack et al. 2010).

From metagenomics surveys it is estimated that 10,000-830,000 species can inhabit each gram of soil (Dance 2008). According to the United Nations Food and Agriculture Organization: "Over 1000 species of invertebrates may be found in a single m^2 of forest soils. Many of the world's terrestrial insect species are soil dwellers for at least some stage of their life-cycle. A single gram of soil may contain millions of individuals and several thousand species of bacteria. A typical, healthy soil might contain several species of vertebrate animals, several species of earthworms, 20-30 species of mites, 50-100 species of insects, tens of species of nematodes, hundreds of species of fungi and perhaps thousands of species of bacteria and actinomycetes. Soil contains the organism with the largest area. A single colony of the honey fungus, *Armillaria ostoyae*, covers about 9 km^2" (FAO 2015).

The scale of threat to biodiversity has never been greater than when genetic engineering can be done at scale in the environment. The efficiency gains now available enable industrial users to do more, more quickly, and to more organisms and species of organisms than ever

before. This capability has released them from having to use a laboratory to compensate for the low efficiency use of modern biotechnology up till now (Heinemann et al. 2023).

A side-effect of this industrial attribute is that the techniques can presently be used by more people in more places (Schwartz 2023). The bottleneck of needing highly trained personnel is removed. Reagents for genome editing which feature in the techniques of synthetic biology are sent to high school children to use in their kitchens (McDonnell et al. 2022) under conditions where unintended exposures are uncontrolled, including those of younger siblings (Heinemann et al. 2023).

This emerging issue of synthetic biology has significant implications for the three objectives of the Convention. Because of ease of use and access to reagents, the threats to biodiversity would be distributed globally and impossible to trace if left unregulated. Regulators would benefit from internationally coordinated horizon scanning and ongoing monitoring and assessment.

III. Priorities for specific further consideration and priorities for better knowledge sharing

In this short submissions we have addressed issues relevant to the list of items from paragraph 6 of decision 15/31. Here we will highlight the need for specific further consideration and better knowledge sharing.

i. Increased development of technologies that genetically modify organisms directly in the field

Under this topic we introduced some of the latest literature for environmental genetic engineering. We discussed how the transition from laboratory containment to outdoor application alters multiple dimensions of risk, by increasing the diversity of exposed species and area of exposure.

Outdoor genetic engineering both increases the number of intended and unintended exposures, it increases the number of exposure pathways. Exposure may be through inhalation, ingestion, and/or contact.

The capacity for field use creates risks that were not contemplated by those who only imagined that any use of modern biotechnology would be contained until an organismal product was made (Heinemann et al. 2023). Already ad hoc regulatory decisions are being taken by individual countries, based on inputs and procedures that may not be robust, and may later be reversed or revised (Heinemann 2019; Heinemann et al. 2023). The level of effort and time required to achieve this regulatory justice is unsustainable in the absence of a coordinating framework under the Convention.

In addition, outdoor (topical) applications, whether or not they result in “permanent” or “transient” phenotypic/genotypic changes, are the basis for broad intellectual property rights claims. These claims extent process-based (utility) type patent protection onto both the exposed organisms and their offspring regardless of heritability or duration of the modification. For example:

“SEVERAL EMBODIMENTS INCLUDE PROGENY SEED OR PROPAGATABLE PLANT PART OF SUCH PLANTS, AND COMMODITY PRODUCTS PRODUCED FROM SUCH PLANTS…WHEREIN THE MODIFICATION OF THE TARGET GENE IS NON-HERITABLE SILENCING OF THE TARGET GENE, OR HERITABLE OR EPIGENETIC SILENCING OF THE TARGET GENE, OR A CHANGE IN THE NUCLEOTIDE SEQUENCE OF THE TARGET GENE; EMBODIMENTS INCLUDE THE DIRECTLY REGENERATED PLANT EXHIBITING MODIFICATION OF THE TARGET GENE AND

> PLANTS OF SUBSEQUENT GENERATIONS GROWN FROM THE DIRECTLY REGENERATED PLANT AND EXHIBITING MODIFICATION OF THE TARGET GENE" (EMPHASIS ADDED TO REF HUANG ET AL., 2018). THE TYPE OF PATENT USED IN THIS CASE IS A UTILITY RATHER THAN PLANT VARIETY PATENT AND EXTENDS TO THE OWNERSHIP OF ORGANISMS AND FUTURE GENERATIONS OF ORGANISMS TREATED WITH EXOGENOUS DSRNA SIMILARLY TO HOW UTILITY PATENTS CLAIM THE USE OF GENETICALLY MODIFIED ORGANISMS…THE MAKER OF THE DSRNA WOULD APPARENTLY OWN AN ORGANISM BECAUSE IT WAS EXPOSED TO THE DSRNA, POTENTIALLY INCLUDING ENTIRE FIELDS OF CONVENTIONAL CROPS OR LONG-LIVED TREES AND THEIR SEEDS THAT HAVE NEVER BEEN MODIFIED BY INSERTION OF DNA. (Heinemann 2019)

The socioeconomic/legal implications of developers acting on these claims are unknown but potentially significant in agriculture, conservation, and even in urban home gardens.

The international regulatory community's approach to assessing the scientific risk and socioeconomic, cultural, and ethical impacts arising from outdoor use of the techniques of modern biotechnology including those used in synthetic biology should be cohesive and coordinated. Continued monitoring and assessment and horizon scanning will help to reduce future trade disruptions and promote products that arise from the responsible use of modern biotechnology/SynBio.

ii. A shift to the development of synthetic biology for environmental, conservation, agricultural and health uses

While we have not made specific reference to this issue, from the range of citations in this submission it is evident that synthetic biology will be applied to all of these areas, either directly or indirectly. For example, pesticidal environmental genetic engineering can substitute the biological imperative of gene drives with the persistence of application by robots in the pursuit of conservation or public health goals.

The boundary between medicine, veterinary medicine, conservation, agriculture, and environment is fluid. Synthetic biology applications that reduce "flu" in chickens could be re-purposed for public health efforts to reduce zoonotic transfer of viral variants to people. Double-stranded RNA therapeutics that target the malaria-causing plasmodium (Cottrell and Doering 2003) could be re-purposed for environmental RNAi pesticides. Either dsRNA or genome editing nucleases that might be used to augment the radiation sensitivity of cancer cells by silencing DNA repair genes (Collis et al. 2003) could be re-purposed in an environmental setting to help control pests using ambient UV radiation. Either application, medical or environmental, could be used to justify the other despite the risk assessments being very different.

Horizon scanning is needed to coordinate and prioritise what may at times be competing environmental, conservation, agricultural, climate, and health goals. In particular, foundational work is needed on how to properly set priorities (Heinemann and Hiscox 2022). Policymakers will require horizon scanning and monitoring to formulate and revise goals that lead to sustainable solutions rather than shifting the problem.

iii. *Increasing sophistication of methods, including, for example, new genome editing techniques, more complex metabolic engineering, the recoding of genomes, and the use of artificial intelligence/machine learning for the redesign of biological systems*

AND Ability to produce new synthetic biomolecules using non-canonical nucleotides and amino acids

We discussed or cited literature on the modification of site-directed nucleases, nucleotides and nucleic acids to achieve a greater range of outcomes (e.g. converting a nuclease used for genome editing into a steric block of translation to cause gene silencing) and stabilities (e.g. chemical modifications of nucleotides to create artificially long intra- or extra-cellular activity times).

Combining the work on digital sequence information (CBD 2018; Heinemann et al. 2018) and cybersecurity (Mueller 2019; 2021) in horizon scanning would be of significant value to the regulatory community and would have additional benefits for the industry. For example, new work suggests that significant errors may routinely arise from incorrect identification of DNA/RNA target sequences (Kwon 2023). That may have limited implications for effectiveness of therapeutics based on reagents tuned to these targets, but important implications for off-target effects if these errors are used in an environmental application.

Furthermore, continued monitoring and assessment of the chemistries for outdoor use should extend to the use of nucleic-acid/protein modification chemistries that alter the persistence or activity of these molecules in the environment or insides cells and organisms.

iii. The use of transient modification of organisms, including, for example, through the use of synthetic double-stranded RNA molecules, nano-particles and genetically modified viruses

We discussed the difficulty of defining "transient" and why whatever time that turns out to be, it is not helpful for risk assessment. Our analysis was supported by the latest research literature that examines how the concept of "transient" is being operationalised (e.g. in-field mutagenesis for genetic engineering) or sometimes commercialised (e.g. null segregants). In this regard, we also drew attention to the use of co-formulants including nanomaterials, or physical abrading, to mechanise the techniques.

We project that the types of products in the research and production pipeline that may be defined as having arisen from transient modifications is large. Concomitantly there is a global effort to control the narrative on this application of the techniques of synthetic biology, portraying them as restorative to a natural state and of no special risk to the objectives the Convention. The premises of that narrative should be thoroughly investigated under the authority of the Convention.

Summary

We have described two broad trends in synthetic biology and identified three important emerging issues. The first emerging issue is that the efficiency of the techniques used in synthetic biology allow their application at scales and in series and combinations that were previously impossible. That capability affects both sides of the risk equation expressed as (probability of harm) x (severity of harm).

The second emerging issue is the tendency to shift any residual oversight of techniques to less relevant hazardous substances legislation and risk assessment. Into the vacuum of appropriate and specific international monitoring and assessment of the use and products of synthetic biology applications as itemised by the AHTEG on Synthetic Biology, sui generis

and inferior approaches will arise. The outcome will be trade disruptions, commercial uncertainty, and most important of all, risks to human health and the environment.

The final emerging issue is that combinations of techniques and the means to use techniques of synthetic biology in the out of doors will lead to an unprecedented socioeconomic challenges for food systems, conservation, and public health initiatives. The immediate challenge arises from predictable and complicated conflicts over IPR claims.

References

Agapito-Tenfen, S.Z.; Okoli, A.S.; Bernstein, M.J.; Wikmark, O.-G.; Myhr, A.I. Revisiting risk governance of GM plants: the need to consider new and emerging gene-editing techniques. Front Plant Sci 2018;9

ASSAf. The regulatory implications of new breeding techniques. Academy of Science South Africa and the Department Science and Technology Republic of South Africa; 2016; https://research.assaf.org.za/handle/20.500.11911/29

Buchholzer, M.; Frommer, W.B. An increasing number of countries regulate genome editing in crops. New Phytol 2023;237:12-15

CBD. Cartagena Protocol on Biosafety. http://www.cbd.int/biosafety/; 2003; http://www.cbd.int/biosafety/

CBD. Synthesis of views and information on the potential implications of the use of digital sequence information on genetic resources for the three objectives of the Convention and the objective of the Nagoya Protocol. AHTEG on Digital Sequence Information on Genetic Resources. Montreal; 2018;

CBD. Synthetic Biology. in: Secretariat Convention on Biological Diversity, ed. CBD Technical Series No 100; 2022; https://www.cbd.int/doc/publications/cbd-ts-100-en.pdf

Chu, P.; Agapito-Tenfen, S.Z. Unintended genomic outcomes in current and next generation GM techniques: a systematic review. Plants 2022;11:2997

Collis, S.J.; Swartz, M.J.; Nelson, W.G.; DeWeese, T.L. Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. Cancer Res 2003;63:1550-1554

Cottrell, T.R.; Doering, T.L. Silence of the strands: RNA interference in eukaryotic pathogens. Trends Microbiol 2003;11:37-43

Dance, A. Soil ecology: what lies beneath. Nature 2008;455:724-725

FAO. Liberation. 2015; http://www.fao.org/fileadmin/user_upload/soils-2015/images/EN/WSDPosters_Promotional_Material/En_IYS_food_Print.pdf

FIFRA. RNAi Technology as a Pesticide: Program Formulation for Human Health and Ecological Risk Assessment. United States Environmental Protection Agency; 2014; http://www.epa.gov/scipoly/sap/meetings/2014/january/012814minutes.pdf

Garbian, Y.; Maori, E.; Kalev, H.; Shafir, S.; Sela, I. Bidirectional Transfer of RNAi between Honey Bee and Varroa destructor: Varroa Gene Silencing Reduces Varroa Population. PLoS Path 2012;8:e1003035

Heinemann, J.A. Should dsRNA treatments applied in outdoor environments be regulated? Environ Int 2019;132:104856

Heinemann, J.A. A scale-based framework for governance and risk of emerging biotechnologies Proceedings of the Global Conference on Green Development of Seed Industries 4–5 November 2021: United Nations Food and Agriculture Organisation; 2022; https://www.fao.org/3/cc1220en/cc1220en.pdf

Heinemann, J.A.; Clark, K.; Hiscox, T.C.; MCCabe, A.W.; Agapito-Tenfen, S.Z. Are null segregants new combinations of heritable material and should they be regulated? Front Genome Ed 2023;4:1064103

Heinemann, J.A.; Coray, D.S.; Thaler, D.S. Exploratory fact-finding scoping study on "Digital Sequence Information" on genetic resources for food and agriculture. Background Study Paper NO. 68. Background Study Papers: United Nations Food and Agriculture Organisation; 2018; http://www.fao.org/3/CA2359EN/ca2359en.pdf

Heinemann, J.A.; Hiscox, T.C. Rethinking the drivers of biotechnologies: a paradigm for holistic climate change solutions. Cur Opin Environ Sustain 2022;59:101222

Heinemann, J.A.; Paull, D.J.; Walker, S.; Kurenbach, B. Differentiated impacts of human interventions on nature: Scaling the conversation on regulation of gene technologies. Elem Sci Anth 2021;9:00086

Heinemann, J.A.; Walker, S. Environmentally applied nucleic acids and proteins for purposes of engineering changes to genes and other genetic material. Biosafety Health 2019;1:113-123

Houri-Ze'evi, L.; Korem, Y.; Sheftel, H.; Faigenbloom, L.; Toker, I.A.; Dagan, Y.; Awad, L.; Degani, L.; Alon, U.; Rechavi, O. A tunable mechanism determines the duration of the transgenerational small RNA inheritance in C. elegans. Cell 2016;165:88-99

Houri-Zeevi, L.; Rechavi, O. A matter of time: small RNAs regulate the duration of epigenetic inheritance. Trends Genet 2017;33:46-57

Krimsky, S. The unsteady state and inertia of chemical regulation under the US Toxic Substances Control Act. PLoS Biol 2017;15:e2002404

Kwon, D. Highly cited genetics studies found to contain sequence errors. Nature 2023;doi: https://doi.org/10.1038/d41586-023-00385-7

Landrigan, P.J.; Fuller, R.; Acosta, N.J.R.; Adeyi, O.; Arnold, R.; Basu, N.; Baldé, A.B.; Bertollini, R.; Bose-O'Reilly, S.; Boufford, J.I.; Breysse, P.N.; Chiles, T.; Mahidol, C.; Coll-Seck, A.M.; Cropper, M.L.; Fobil, J.; Fuster, V.; Greenstone, M.; Haines, A.; Hanrahan, D.; Hunter, D.; Khare, M.; Krupnick, A.; Lanphear, B.; Lohani, B.; Martin, K.; Mathiasen, K.V.; McTeer, M.A.; Murray, C.J.L.; Ndahimananjara, J.D.; Perera, F.; Potočnik, J.; Preker, A.S.; Ramesh, J.; Rockström, J.; Salinas, C.; Samson, L.D.; Sandilya, K.; Sly, P.D.; Smith, K.R.; Steiner, A.; Stewart, R.B.; Suk, W.A.; van Schayck, O.C.P.; Yadama, G.N.; Yumkella, K.; Zhong, M. The Lancet Commission on pollution and health. Lancet 2018;391:462-512

Li, J.; Zhao, H.; Zheng, L.; An, W. Advances in synthetic biology and biosafety governance. Front Bioeng Biotechnol 2021;9:598087

McDonnell, L.; Moore, A.; Micou, M.; Day, C.; Grossman, E.; Meaders, C. CRISPR in your kitchen: an at-home CRISPR kit to edit genes in Saccharomyces cerevisiae used during a remote lab course. J Microbiol Biol Educ 2022;23:e00321-00321

Mitter, N.; Worrall, E.A.; Robinson, K.E.; Li, P.; Jain, R.G.; Taochy, C.; Fletcher, S.J.; Carroll, B.J.; Lu, G.Q.; Xu, Z.P. Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. Nat Pl 2017;3:16207

Mueller, S. Are market GM plants an unrecognized platform for bioterrorism and biocrime? Front Bioeng Biotechnol 2019;7

Mueller, S. Facing the 2020 pandemic: what does cyberbiosecurity want us to know to safeguard the future? Biosafety Health 2021;3:11-21

Murphy, K.A.; Tabuloc, C.A.; Cervantes, K.R.; Chiu, J.C. Ingestion of genetically modified yeast symbiont reduces fitness of an insect pest via RNA interference. Sci Rep 2016;6:22587

Nance, K.D.; Meier, J.L. Modifications in an emergency: the role of N1-methylpseudouridine in COVID-19 vaccines. ACS Cent Sci 2021;7:748-756

Novick, A.; Weiner, M. Enzyme induction as an all-or-none phenomenon. Proc Natl Acad Sci USA 1957;43:553-566

Palauqui, J.C.; Elmayan, T.; Pollien, J.M.; Vaucheret, H. Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J 1997;16:4738-4745

Parker, K.M.; Borrero, V.B.; van Leeuwen, D.M.; Lever, M.A.; Mateescu, B.; Sander, M. Environmental fate of RNA interference pesticides: adsorption and degradation of double-stranded RNA molecules in agricultural soils. Envir Sci Technol 2019;53:3027-3036

Rang, A.; Linke, B.; Jansen, B. Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. Eur Food Res Technol 2005;220:438-443

Schwartz, L. Edited human DNA at home with a DIY CRISPR kit. Motherboard Tech by Vice; 2023; https://www.vice.com/en/article/qjkykx/diy-crispr-gene-editing-kit-human-dna?utm_medium=email&utm_source=rasa_io&utm_campaign=newsletter

Sharma, V.K.; Marla, S.; Zheng, W.; Mishra, D.; Huang, J.; Zhang, W.; Morris, G.P.; Cook, D.E. CRISPR guides induce gene silencing in plants in the absence of Cas. Genome Biol 2022;23:6

Sirinathsinghji, E. Transferring the laboratory to the wild: An emerging era of environmental genetic engineering. Biosafety Briefing: Third World Network; 2019; https://biosafety-info.net/wp-content/uploads/2019/11/Biosafety-briefing_From-lab-to-wild.pdf

siRNAmod. A database of experimentally validated chemically modified siRNAs. 2018; http://crdd.osdd.net/servers/sirnamod/index.php

Solomon, S.M. Genome editing in animals: why FDA regulation matters. Nat Biotech 2020;38:142-143

Touzdjian Pinheiro Kohlrausch Távora, F.; de Assis dos Santos Diniz, F.; de Moraes Rêgo-Machado, C.; Chagas Freitas, N.; Barbosa Monteiro Arraes, F.; Chumbinho de Andrade, E.; Furtado, L.L.; Osiro, K.O.; Lima de Sousa, N.; Cardoso, T.B.; Márcia Mertz Henning, L.; Abrão de Oliveira Molinari, P.; Feingold, S.E.; Hunter, W.B.; Fátima Grossi de Sá, M.; Kobayashi, A.K.; Lima Nepomuceno, A.; Santiago, T.R.; Correa Molinari, H.B. CRISPR/Cas- and topical RNAi-Based technologies for crop management and improvement: reviewing the risk assessment and challenges towards a more sustainable agriculture. Front Bioeng Biotechnol 2022;10

USDA. Soil Health Nuggets. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1101660.pdf

Vastenhouw, N.L.; Brunschwig, K.; Okihara, K.L.; Müller, F.; Tijsterman, M.; Plasterk, R.H.A. Long-term gene silencing by RNAi. Nature 2006;442:882-882

Whangbo, J.S.; Hunter, C.P. Environmental RNA interference. Trends Genet 2008;24:297-305

Windels, P.; Taverniers, I.; Depicker, A.; van Bockstaele, E.; De Loose, M. Characterisation of the Roundup Ready soybean insert. Eur Food Res Technol 2001;213:107-112

Womack, A.M.; Bohannan, B.J.M.; Green, J.L. Biodiversity and biogeography of the atmosphere. Phil Trans R Soc B 2010;365:3645-3653

Zeng, X.; Jiang, H.; Yang, G.; Ou, Y.; Lu, S.; Jiang, J.; Lei, R.; Su, L. Regulation and management of the biosecurity for synthetic biology. Synth Syst Biotechnol 2022;7:784-790

Zhang, L.; Hou, D.; Chen, X.; Li, D.; Zhu, L.; Zhang, Y.; Li, J.; Bian, Z.; Liang, X.; Cai, X.; Yin, Y.; Wang, C.H.; Zhang, T.; Zhu, D.; Zhang, D.; Xu, J.; Chen, Q.; Ba, Y.; Liu, J.-J.; Wang, Q.; Chen, J.; Wang, J.; Wang, M.; Zhang, Q.; Zhang, J.; Zen, K.; Zhang, C.-

Y. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 2012;22:107-126

Zhang, R.; Xu, W.; Shao, S.; Wang, Q. Gene silencing through CRISPR interference in bacteria: current advances and future prospects. Front Microbiol 2021;12