Synthetic biology

– biosafety and contribution to addressing societal challenges

November 2016

genøk

Biosafety Report 2016/02
GenØk - Centre for Biosafety, Tromsø, Norway
December 2016

Synthetic biology – biosafety and contribution to addressing societal challenges

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Introduction

Synthetic biology has become increasingly important as an approach and a tool to solve societal challenges, such as replacing fossil fuel, and to develop more efficient biological compounds, new applications for bioremediation, a platform for more efficient vaccine intervention and to improve drug targeting. As the potential of the technology and the scope of products and organism created by synthetic biology have emerged, it is clear that also biosafety research and regulation of synthetic biological products need careful consideration.

The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) is an international regulatory instrument concerning the safe transfer, handling and use of living modified organisms (LMOs) resulting from modern biotechnology. The objective of the CPB is to provide an adequate level of protection from potential adverse effects on the conservation and sustainable use of biological diversity, also taking into account risks to human health.

During the conference of the parties to the CBD (COP 12) in October 2014, the Parties were urged to take a precautionary approach, and to establish effective measures to regulate the environmental release of any organisms, components or products resulting from synthetic biology techniques. Parties were also urged to conduct appropriate risk assessments before approving field trials of organisms resulting from synthetic biology techniques. Moreover, that synthetic biology organisms, components and products should be subject to scientific assessments that consider risks to conservation and sustainable use of biodiversity, as well as human health, food security and socio-economic considerations.

The scope of this report is to give an introduction to synthetic biology, the research and applications, and the biosafety questions. The potential to learn from risk assessment of GMOs and the adequacy of current risk assessment methodologies are discussed and potential ethical and socio-economic by synthetic biology-based products and applications are adressed. Relevance and application of the Cartagena Protocol and international law to synthetic biology are presented together with a suggestion for a regulatory approach to synthetic biology. The sources of information in this report is scientific literature from peer-reviewed articles and reports, in addition a special emphasis has been put on issues, options, and challenges identified and discsussed by participants in two courses held by GenØk – Centre for Biosafety in collaboration the Norwegian Institute for Bioeconomy Research (NIBIO) in 2015 and 2016 and local partners North West University and Bogor Agricultural University.

The two courses entitled *Capacity building course: Synthetic biology – biosafety and contribution to addressing societal challenges*, were organized with participants from the regions of southern Africa (organised in Potchefstroom, South Africa) and the ASEAN countries and China (organised in Bogor, Indonesia). 75 participants from 21 countries participated, which illustrates the huge interest and need for courses that teaches potential positive outcomes as well as challenges related to the use and introduction of synthetic biology.

Finally, we would like to thank the Norwegian Ministry of Foreign Affairs for taking initiative, supporting and funding of the project, of which the outcome is two courses and this report.

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Synthetic Biology: Science, Experimental Approaches and Applications in Biotechnology

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Synthetic biology – introduction and historical perspectives

The invention of recombinant DNA technology in the seventies marked a shift from classical to molecular biology. The possibility to genetically engineer cells and organisms has an immediate impact on biotechnology with the recombinant production of insulin and human growth hormone by using bacteria as production systems. The recombinant DNA technology developed fast and contributed top accelerated understanding within cell biology and biochemistry. Beyond 1990 the development exploded by in particular new DNA sequencing technologies, and later a boost of so-called omics technologies, and bioinformatics appeared and rapidly become very central. Molecular biology developed fast by introducing new and advanced heavy infrastructures and high-through-put generation of bid data. Around year 2000 Systems biology was introduced as a new research field taking this new and holistic view into focus by integrating big data with mathematical modelling, aiming at predicting and understanding all cell functions at one time. A few years later synthetic biology appeared as a complementary filed to systems biology, taking the design and engineering aspects of molecular biology to new levels. Since then, synthetic biology has become a key filed in molecular biology, rapidly pushing the limitations for genetic engineering both in complexity and speed, to new levels. It has become an important tool for generating new basic science and with many applied aspects. So far the largest progress has been om microorganisms, and this report will naturally have focus on synthetic biology on bacteria.

Synthetic biology – synthetic life

On the 20th of May 2010 the J. Craig Venter Institute (JVCI) in US announced that they had created synthetic life in the laboratory. They had constructed the first self-replicating synthetic bacterium and named it *Mycoplasma mycoides* JVC-syn1.0; Synthia. This represented historical breakthrough in a series of cutting edge research from this group; in 2003 they constructed the first synthetic virus; in 2008 they constructed the first complete synthetic chromosome; in 2009 they performed the first "microinjection" of a complete chromosome into bacterial cell; and in 2010 they combined these technologies to creation of Synthia. The Synthia chromosome is composed of more than 1 million base pairs and represents the biggest chemical molecule made on laboratory. The development of Synthia required development of completely new methodologies to make and handle large DNA molecules; these will be presented and discussed below. As Craig Venter stated: *this is probably the first living creature on this planet whose parent is a computer.* Synthia represented a shift from where researchers traditionally only could read the genetic code into an era where they can write it.

Synthia received enormous global attention, and not only in the academic circles. Some with enthusiasm and others with fear. It seriously put the field synthetic biology on the map as an enabling technology for both basic research and with many obvious applied perspectives. Craig Venter claimed to have to major goals with this new technology; one was to create a minimal living bacterial cell stripped for all unnecessary genes (basic science), and the second goal is to use this

technology to create next generation cell factories for industrial biotechnology purposes (applied science). In the years past there has been new great advances in genetic engineering technologies, including methods for synthesizing, cloning and handling large DNA molecules. Synthetic biology can be defined as *engineering of biology: design and construction of new biological components and systems with new functions for useful purposes*. Synthetic biology has become a central field with great impact on both fundamental life science and with applied perspectives.

Why did synthetic biology appear – how did it start? Synthetic and biology are two words that do not fit well together; synthetic means something artificial or artificially made, while biology is science of life and living nature. Many researchers ask; is Synthia truly synthetic? Have they truly created synthetic life? The fact is that Synthia is made of organic materials just as any natural bacterial cell and a more correct description (and as specified by Venter himself) is that this is a living bacterium based on a chemically synthesized genome that was designed and written by the scientist.

In many ways synthetic biology is a logic continuation of genetic engineering by utilizing todays great advances in recombinant DNA technologies including DNA synthesis, DNA sequencing, omics technologies, new cloning technologies, bioinformatics, and systems biology. Professor Victor de Lorenzo from University of Madrid stated that what is fundamentally new with synthetic biology is that bioengineering has developed from being a metaphor to a methodology: *The central dogma of molecular biology was that DNA makes RNA and RNA makes protein – now the vision of synthetic biology is that parts make devices and devices make systems*. Biotechnology is undergoing an exponentially accelerating development, in many aspects to what we today experience in data and informatics technology, and synthetic biology is a major driving force in this progress.

New possibilities open up with synthetic biology

The largest advances within synthetic biology has so far been on microorganisms, bacteria and yeast. Bacteria are unicellular organisms, and over time a spectre of methods have been established enabling advanced genetic modifications of bacteria. Moreover, bacteria are easy to cultivate on the laboratories, they have short (down to 10 minutes) generation time, and there are few ethical considerations associated by altering them genetically (as compared to higher animals). Bacteria has for decades been used as cell factories (**Figure 1**) in biotechnology for sustainable production of a wide range of industrial (e.g. enzymes, biopolymers, biofuels, biomaterials) and medical / pharmaceutical (e.g. regenerate medicines, vaccines, antibiotics, antibodies, vitamins, cytokines, antioxidants) products, as well as components and ingredients for food and feed (e.g. amino acids, fatty acids), cosmetics (e.g. sun protectants, components in skin and hear products), diagnostics and environment (e.g. biosensors, bioremediation). We will see more in detail on microbial cell factories in section 3.

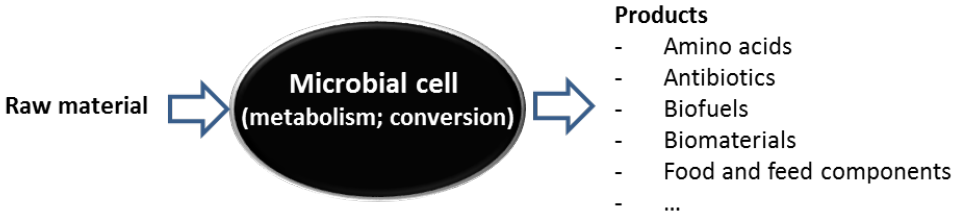

Figure 1. Synthetic biology is a powerful technology to construct microbial cell factories for applications in white biotechnology

Today – in the bio economy era - there is a major global transition from traditional chemical industry to biotechnological processes; this has both economic and environmental consequences. In this industrial biotechnology - often also termed white biotechnology – synthetic biology is expected to have a huge impact in the years to come. BCC Research is a leading market analysis company specifically on changes driven by science and technology; they estimate the synthetic biology sector will grow 11 Billion USD within 2016 and they expect major investments in this field in the next years. McKinsey & Company estimate that this technology will grow to 100 billion USD within 2025. Thus, expectations to synthetic biology are high.

Important technologies beyond the development of synthetic biology

DNA sequencing was first demonstrated in 1976 by Alfred Sanger and the method he developed – the Sanger method – was the standard used and gradually defined for many years. One crucial event that caused major impact on development of DNA sequencing technologies was the Human genome project initiated as an international consortium HUGO in 1990. The goal – which at that time was very visionary and ambitious – was to sequence the human genome. Around 1997 the US pharmacy company Celera took up competition with HUGO and they introduced the so-called shut gun sequencing technology – aiming at completing the human genome sequencing faster and then to patent the results. A race was started eliminating in an agreed co-publication in Science and Nature of the human genome sequence first in 2001, and a more complete version in 2003. Shotgun sequencing was faster and cheaper than Sanger sequencing, and in parallel with the human genome a number of bacteria and yeasts were also genome sequenced. Today, many years later – the completion of HUGO more than representing the end of a giant project represented the start of a new era of genomics. Next generation DNA sequencing technologies, such as Pyrosequencing, 454, Illumnia, SOLiD, and Single molecule sequencing, have developed at high speed and today virtually everything is being sequenced fast and at low prize. In total, DNA sequencing has completely revolutionized biotechnology and had a major impact on development of synthetic biology as we know it today. More organisms are explored, and more genes and gene clusters are characterized, all highly useful for advanced genetic engineering. And with genomics followed massive developments in the omics technologies, including transcriptomic, proteomics, metabolomics, fluxomics, phonemics, and others – all together highly useful tools for advanced synthetic biology.

Another technology with high impact on synthetic biology is template free DNA synthesis (i.e. by not using PCR). Today, companies offer such services; however – compared to DNA sequencing the development of DNA synthesis technology has been slow. There are clear limitations to the size of DNA molecules that can be synthesized and prizes are still relatively high. For example, when the Synthia chromosome was chemically made, they used DNA synthesis to generate in in a high number of small pieces, and then sophisticated methodologies to link them together in a fixed determined order eventually generating complete chromosome. It is expected that synthetic biology development will make a large jump if DNA synthesis technology is dramatically improved.

Finally, new and improved cloning techniques for large – really large – DNA molecules have been of major importance for developments in synthetic biology such as;

- Gibson assembly
- Circular polymerase extension cloning (CPEC)
- Golden Gate

- Multiplex automated genome engineering (MAGE)
- Conjugative assembly of genome engineering (CAGE).

Today genetic engineering in bacteria can be executed at any levels from single genes on small plasmids, to BAC vectors with several 100 kb large inserts, to entire chromosomes. Moreover, robotic laboratories make it possible to work both large and fast, enabling high-through put generation of large libraries of genetically engineered organisms.

Synthetic biology has ethical and societal aspects

Synthetic biology is also associated with aspects related to biosafety, bioterror and even biological weapons. Clearly one can imagine that this technology can be misused for bad intentions to create pathogenic viruses and bacteria to be used in terror and war. As an example, researchers at an US University managed to create a synthetic human pathogenic influenza virus on the laboratory – the aim was to publish (this was open) and to demonstrate the huge potential of this technology for such directions. It is also argued that synthetic biology has made biotechnology research crossing a border; we are playing with nature – playing god; what will be the next - an and will it be used to improve humans? Today, civilized countries more and more require that governmentally funded synthetic biology projects should include Ethical, Legal and Societal Aspects (ELSA) and later also Responsible Research and Innovation (RRI), to create a balance between in the possibilities and potential on then one side and the risks and the ethical parts on the other side. Examples of issues and questions discussed are:

- Do the researchers have control on what they are constructing?
- Creating organisms that behave as predicted – contradictory to biology?
- Biosafety; undeliberate and unknown effects? Treat to human health and environment?
- Religious aspects - playing God?
- The precautionary principle?
- Do we need this technology? Is the world better without?
- Do we need new rules and laws for bio patenting?
- Good purposes:
    - healthcare, medicine, bioenergy, remediation, food, vaccines, new materials
- Bad purposes:
    - Undeliberate release of GMOs in nature; consequences?
    - Biohackers – are we losing control?
    - Bioterror – synthetic pathogens; biological weapons

More and more there is an understanding that the education of responsibilities of researchers in the field is important to ensure that this powerful technology is used for the good purposes; to generate basic knowledge in life science as well as to generate innovations for a sustainable development. An interesting parallel to all this is the UK Synthetic Biology Dialogue (2010) where the UK research council published findings from a public dialogue around synthetic biology. The following important questions should be considered for synthetic biology proposals

- What is the purpose?
- Why do you want to do it?
- What are you going to gain from it?
- What else is it going to do?
- How do you know you are right?

In particular, the latter two question are interesting as they really force the researcher to speculate deeper with respect to the expected results from their proposed synthetic biology research.

Designing synthetic biological organisms - processes, techniques and utilization

Since early in the 2000th several large transcontinental centres for synthetic biology were established in the US where universities and industries go together with common goal on how to make biology easier and safer to engineer. SynBerc (http://www.synberc.org/) was started in 2006 aiming at paving the way for synthetic biology by:

- Developing basic knowledge and tools useful for design and construction of biological systems that can perform complex operations;
- Educating and train next generation for synthetic biology;
- Developing best practice for safe and ethical responsible development of synthetic biology.

In this period, the analogy between biological and mechanical systems appeared, and new terminologies such as bio bricks, standardized parts, the principle of orthogonality, robotized cloning, gene depositories, where introduced. For example, bio bricks and standardized parts describes specific DNA- sequences with precisely defined properties, such as genes, promoters, ribosome binding sites, and terminators. These bio bricks can then be assembled in different ways to construct devices with predictable functions. In reality, scientist however now that this is a vison far away from reality as few or no single DNA elements can be completely functionally described and that the function is highly and unpredictable dependent on the context. We will look more into this in section 3 below.

To main approaches of synthetic biology: top-down and bottom-up

The famous quote by Nobel Prize Laureate Professor Richard Feynman: *what I cannot create – I do not understand*, has often been used as a motivation for synthetic biology as an important tool to generate basic knowledge in life science. It combines genetic design with genetic engineering to modify and construct biological systems and then evaluate the effects of the genetic modifications. There are two principally different approaches for synthetic biology, denoted top-down and bottom-up (**Figure 2**).

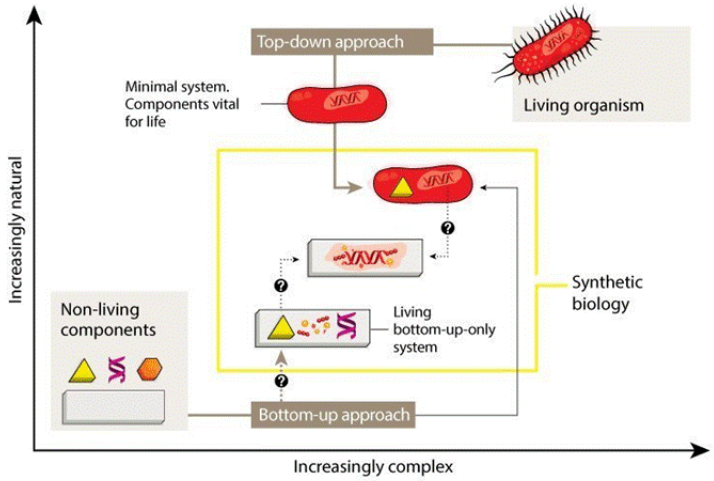

Figure 2: Top-down vs the bottom-up approach for synthetic biology along axes of increasingly natural (y-axis) and increasingly complex (x-axis).

The creation of Synthia (see above) represented a so-called bottom-up approach; the chromosome was designed and assembled from single components into the construction of the entire cell. In principle, this technology can be used to generate cells with new and predicted properties previously not found in nature. In reality, even today 6 years after, this technology is very tedious and not commonly used in synthetic biology research, although technologies for chemical synthesis of chromosomes is gradually improving. A much more commonly used alternative is the top-down approach where a natural cell is used as a host (commonly denoted *chassis*) for the engineering of new biological functions into it. The latter is technically less challenging and the level of complexity can be gradually increased. In addition, the latter strategy can take advantage of the huge amount of accumulated biological knowledge of the host organism, into the successful engineering of synthetic organisms with new properties. However, it should be stated that it is an open question which of these two approaches will be the most important for synthetic biology development in the future; technology developments will likely play a major role as well. Regardless the approach used –the overall aim of all synthetic biology research remains the same: to create new biological systems that do not exist in nature.

From metabolic engineering to systems biology and synthetic biology

Metabolic engineering was introduced for the first time in the scientific community in 1992 and defined as the purposeful modifications of cell metabolism by using recombinant DNA technology. Obviously, the ideas behind metabolic engineering has been inherited in synthetic biology as we know it today; the main difference is the enormous technology developments and general biological knowledge that has moved design, modelling and engineering to completely new levels of complexity. One important and complementary field is systems biology which combines big experimental omics' data with advanced mathematical modelling to describe and quantify complex biological systems including entire cells. Systems biology aim to understand how all components in a cell collaborate to maintain life. While systems biology is regarded as analytical science, synthetic biology is regarded as operational science; it makes sense then that combining these two sciences is powerful to engineer and create synthetic cells in a rational and predictable manner. The synergy between metabolic engineering, systems biology and synthetic biology is illustrated in **Figure 3**.

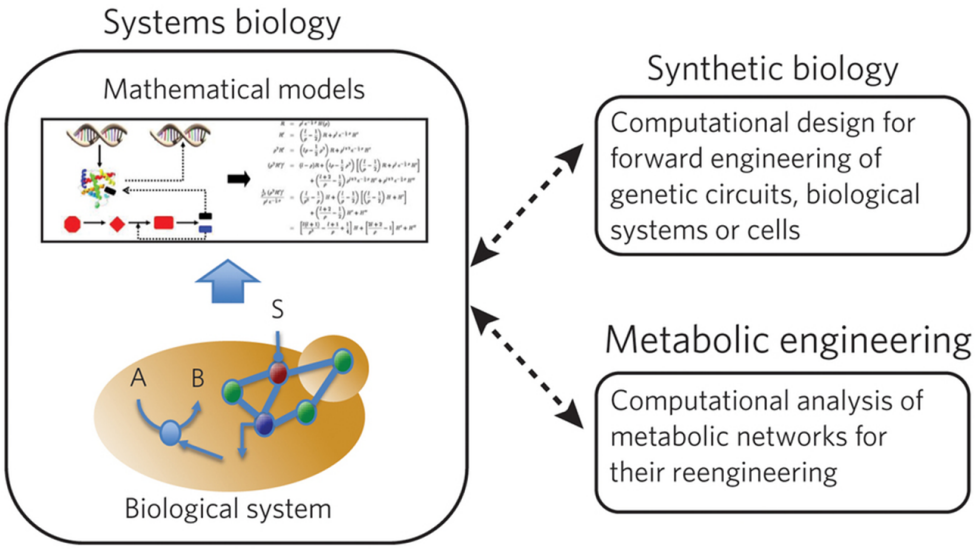

Figure 3. Metabolic engineering and synthetic biology are operational sciences and rely on systems biology modelling of cell functions for design and evaluation.

Synthetic biology experiments; examples

As mentioned above, bacteria have for decades been used as cell factories in white biotechnology for sustainable production of a wide range of chemicals, polymers and proteins for uses in industry, food and feed, pharmacy, and medicine. Experimental synthetic biology is largely about advanced genetic engineering, cloning, and recombinant DNA technology, and White biotechnology plays a vital role in the bio economy. The bacterium *Escherichia coli* is easy to genetically modify and therefore typical host for the genetic engineering work; engineering can be on the chromosome and/or by using self-replicating plasmids (**Figure 4**).

There are several advantages by using bacteria as cell factories for bioprocess, such as:

- Can be cultivated to high cell densities in large contained bioreactors
- They are easy to genetically modify and understand
- Accumulated knowledge on biochemistry, physiology, metabolic network models
- Easy to destruct
- Less energy demand and less by products compared to chemical synthesis
- New products

Bacteria naturally synthesize a number of products commercially in use such as antibiotics, pigments, antioxidants, enzymes and biopolymers. By using synthetic biology, we can improve production capabilities, we can make them able to produce new compounds and we can modify cells to utilize alternative organic biomass raw materials (lignocellulose, methane, methanol) for growth. Compared to chemical synthesis, using bacteria as biocatalysts are much more environmentally friendly (lower temperatures, less waste products, cheap and renewable raw materials) in line with bio economy ambitions.

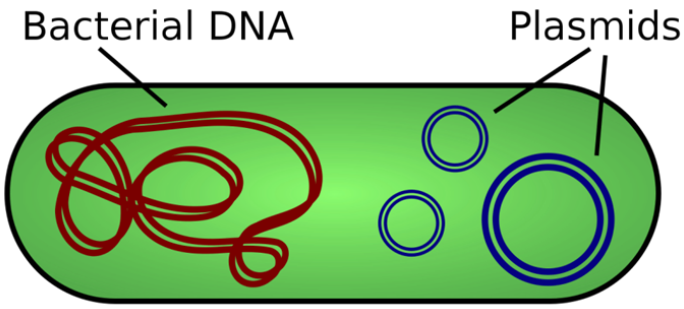

Figure 4. The bacterial cell has one circular chromosome and plasmids; both can be subjected to genetic engineering in synthetic biology.

Different strategies have successfully been applied to construct superior cell factories by using synthetic biology, and we here show three examples of commonly used strategies, as outlined below: ***Construct a synthetic pathway to be expressed in a bacterial chassis for production of unnatural chemical.*** This is a very much used strategy both to improve production of a natural product as well as to make a bacterium produce a new compound. Typically, multiple versions of the same synthetic pathway are constructed in parallel to optimize it with respect of using different combinations of promoters, ribosome binding sites, terminators as well as different homologous versions of the pathway genes.

An outline of the overall strategy is as follows:

- Genetic engineering / cloning work typically done in plasmid in E. coli
- Transform hybrid plasmid to host bacterium (chassis)
    - Alternative 1: Plasmid remains in host and synthetic pathway expressed from it
    - Alternative 2: Synthetic pathway integrated in host chromosome; plasmid lost
- Analyse constructed bacterial strain for the desired new properties

Controlled knockout of genes in the bacterial chassis. Genetic engineering is a combination of adding, modifying and removing genes. Gene knockouts are important to pull the carbon flux towards the desired product or precursors, limiting side product formation that cost both carbon and energy. Also, genes encoding well expressed host proteins with no important biological functions may be deleted for same host optimization reasons. Several methodologies s exist for genome engineering / editing, and recently also efficient methods for several simultaneous gene knockouts have been established, e.g. MAGE and Crispr/cas9.

Combining controlled knockout and expression of synthetic pathway. Advanced synthetic biology typically needs a combination of the two strategies above; side-branches and other cellular functions taking carbon and/or energy away from the desired product are knocked out and synthetic pathways are engineered and integrated into the optimized bacterial chassis. It is generally useful to work sequentially and evaluate and refine engineering at the different stages to be sure that the predicted effects truly work.

Microbial cell factories

One famous example is the synthetic yeast cell constructed by Professor Jay Keasling in US that could synthesize the important anti-malaria drug artimisinin from simple sugar raw materials. Totally 13 heterologous genes were recruited and assembled into a complete synthetic artimisinin pathway in the yeast cell. Keasling has later explained that the design of the synthetic cell was made in weeks and months while it took more than 150-person man's research years to make it work. As Keasling stated: *analogous to the design of chemical manufacturing facilities, the flow of chemicals through enzymatic reactions within a cell must be optimized within the context of other cellular processes to ensure product composition and to minimize the generation of undesirable products*. This very important experiment clearly also demonstrated that the term standardized parts can be questioned when it comes to genetic elements; the success of this work very much was a matter of tedious trial and error.

Several successful examples describe the construction of synthetic cell factories for production of complex chemicals such as biofuels, antibiotics and carotenoids by constructing and integrating completely synthetic pathways into biotechnologically well-established bacterial hosts such as E. coli, analogous to the Keasling approach above. Common in literally all cases is that the scientific focus very much lies in the design and optimization of the synthetic pathways enabling balanced and proper expression of the heterologous genes into functional biosynthetic pathways in the heterologous host.

Conclusions and Perspectives

Synthetic biology is a multi-disciplinary science with strong basis in metabolic engineering and systems biology, and it has both basic and applied aspects. It is expected to play major role in medical and industrial biotechnology in the coming years and the economic estimates on a global scale are huge and optimistic. Two principally different approaches – top-down and bottom down – are in use and so far the top-down approach using well established microbial hosts as chassis dominates. The major scientific challenges are the standardization and design of biological components and devices – to get things working in a heterologous environment. Experience from synthetic biology show that we are still today far from the vision of standardization and the principle of orthogonality, and synthetic biology should be performed in line with systems biology understanding of entire cell functions to be successful. Synthetic biology has great opportunities and potentials and at the same time it raises societal, ethical and legal questions. Technology developments will play a major role in future development of synthetic biology.

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Synthetic Biology and Biosafety

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Regulatory status of Synthetic biology and the need for biosafety research

Regulation of synthetic biology and more prominent, organisms created by genome editing, has emerged as an area of conflicting viewpoints. The US Food and Drug Administration (FDA) has taken the viewpoint that gene edited organisms should not be regulated while the official European viewpoint is still not clarified. There are considerable international efforts through the Convention of Biological Diversity (CBD), to collectively define and clarify the regulatory status of synthetic biology and organisms and products created thereof. Although this is a relatively recent initiative, the research and construction of synthetic organisms and products are not a new one. Synthetically produced pharmaceuticals, cosmetics, food additives and biofuel components have been around for some time, but new technological advancements have pushed the applications from microbes in fermenters into possible deliberate released products (i.e in husbandry or agriculture). With these new products, mainly but not exclusively made by gene editing, developing risk assessment recommendations and procedures are crucial for minimizing risk for negative impact on the environment and human/animal health. As the following text show, the great potential for solving societal challenges by utilizing synthetic biology must be combined with scientifically sound biosafety research as there naturally many knowledge gaps that needs to be investigated. The case-by-case and step-by-step approach recommended in risk assessment of genetically modified organisms (GMOs) is highly relevant to synthetic organisms and therefor, much of the current unanswered question are not connected to specific products, but to general observations of the individual approaches to synthetic biology (i.e. top-down, bottom-up processes).

The European regulatory system for GMO approval – usefulness for Synthetic Biology?

In EU, the European food safety authority (EFSA) evaluates the safety of every GMO before they are approved to be put on the marked. This includes the usage as food and feed as well as cultivation and encompasses GM animals, plants and microorganisms (www.efsa.europa.eu). After the recommendation from EFSA, which includes input from member states, the final decision is being made by the EU commission´s standing committee on plants, animals, food and feed. As long as synthetic biology products and organisms falls under EU regulation, it is likely that the same or a very similar risk assessment and risk management procedure will be installed also for these. There is an abundance of recommendations (published by OECD, European food safety authority (EFSA), European Commission, CODEX etc.) for risk assessments regarding GMOs, and these may also be useful starting point for requirements for risk assessment of synthetic biology products and organisms. For instance, requirements for molecular data, description of modification, environmental impact, toxicity and allergenicity are all relevant steps in assessment of synthetic organisms intended for deliberate release.

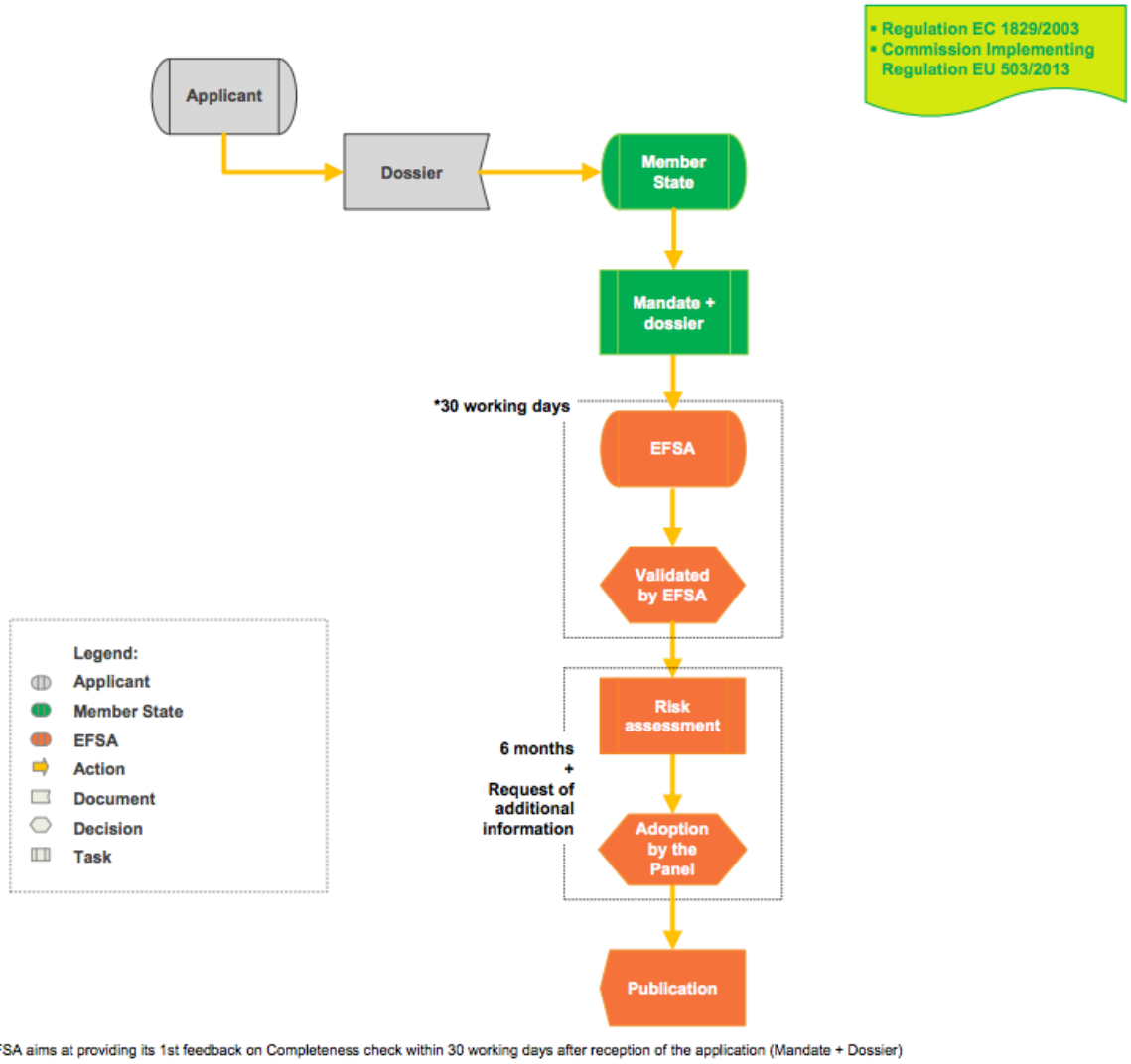

***Figure 1**: The process of risk assessment and approval of applications of GMOs in EU. https://www.efsa.europa.eu/sites/default/files/assets/apdeskapplworkflowgmo.pdf*

As of fall 2016 there are not yet any international agreed definition or risk assessments procedures on organisms and/or products derived from synthetic biology, but several reports and opinions exists. For instance, the EU Commission has released three opinions on synthetic biology and the secretariat on the Convention of Biological Diversity (CBD) has released a comprehensive report on synthetic biology focusing on the potential impact on biodiversity as well as gaps and overlaps with the provisions of the Convention and other agreements.

Synthetic Biology approaches and biosafety concerns

For biosafety purposes, it is useful to divide synthetic biology into 5 different categories, each with its own specific biosafety questions and knowledge gaps. It is important to stress that the biosafety questions of these categories are of a general nature and a case by case approach is necessary to adequately address specific products or organisms.

1. *DNA circuits/Biobricks – pathway engineering*

DNA circuits are also known as what is referred to as biobricks. The idea is to choose and combine DNA in a modular fashion to perform the desired function in a predictable manner. Websites and communities like http://parts.igem.org, promotes this way of building new functions into living systems. The registry of biological parts facilitates the sharing of these biobricks between members of the online community that can pick and combine the individual components to assemble into new DNA circuits.

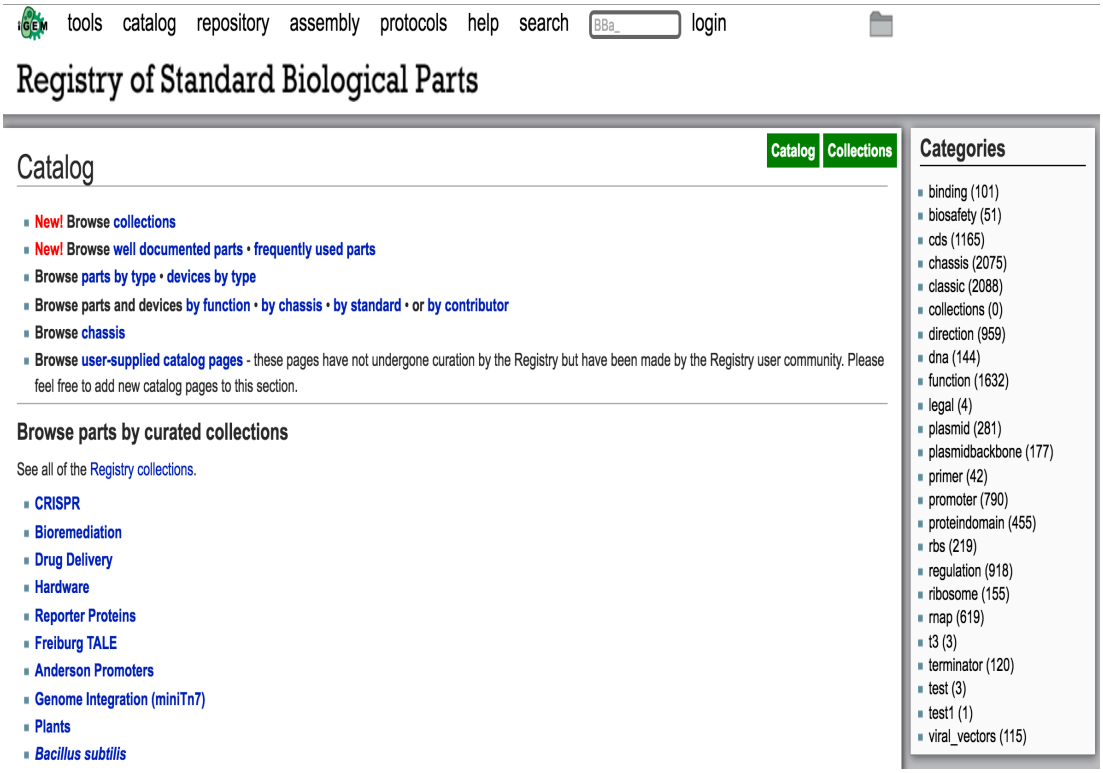

***Figure 2**: igem registry of standard biological parts website showing some categories of biobricks described in the registry. Source: parts.igem.org/Catalog*

Biosensors, production of new biomaterials and food additives/replacements as well as biofuel components are some of the applications developed from biobricks. Examples like liquid hydrocarbons (Monoterpenes), that can be produced in yeast and used in a wide range of applications from plant protection, fragrance to replacement jet fuel (e.g. Brennan et al., 2015), shows the great potential and flexibility of synthetic biology to develop novel products.

Even though regulation of synthetic biology and products thereof, are not clear yet, there are a number of biosafety questions already emerging. Specifically for biobricks, a great deal of concern is connected to the fact that biological systems are complex and that biobricks usually contains more than one inserted/modified gene. If we compare to GMOs, the most similar would be GMOs with multiple genes inserted – so called stacks or smartstacks. The basis for risk assessment of GMOs with stacked genes are a comparative approach with the closest unmodified variety (or parent), strengthened with prior risk assessments of individual genes combined in the stacks. For instance, a plant that contain a herbicide tolerant combined with a insecticidal trait, would be based on the risk assessment of the herbicidal trait alone and the insecticidal trait alone. The risk assessment of the

stack is made easier on the basis of the "history of safe use" for the individual traits. For biobricks, where multiple genes are assembled into a new pathway, there are no risk assessments available for the individual genes. In addition, the closest comparable variety or organism may be very different and therefor large differences may be expected on gene and protein expression as well as on metabolic analysis.

The registries of biological parts, like igem, have just a minimal set of descriptors related to the deposited parts. These are features like DNA length, sequence and compatibility with other parts in the registries. Understanding potential risks are made difficult because it is a complete lack of description of properties of the novel registered components. Coupled with a lack of history of use, the lack of properties hampers the understanding of potential environmental effects of an accidental or an intended release. From a biosafety standpoint, increasing the requirements for submission into the registries to include basic properties, would be welcomed to better understand potential direct effects or combinatorial effects of parts with synergistic properties.

2. Pathway engineering and deliberate release

A potential huge environmental benefit from organisms produced by synthetic biology are microorganisms designed for bioremediation. Oil spills and metal pollution from mining industry could potentially be cleaned up by using microorganisms that are designed to digest the specific pollutant, hence reverting the environment back to the natural pre-pollution state. These organisms will of course be designed for release and to survive in the environment, at least until their task is fulfilled. Essential environmental biosafety concerns for organisms designed for deliberate release, are to understand their potential for survival, growth and spread in-, and outside, the intended biotic conditions. What happens to the organisms once the pollution is cleaned up? How huge biomass and hence DNA, will be accumulated and thereby be a source for selection of mutants allowing spread outside the environment and also a source for potential horizontal gene transfer?

3. Genome minimization (top down)

Genome minimization is an approach to understand basic evolutionary biology and a tool for maximizing the efficiency of the applied biotechnology process. For instance, the cost-efficiency of yeast cells producing a synthetic compound may be dramatically increased by minimizing the genome so that only cell-processes relevant for production of the compound are turned on. That way, all the cells accumulated energy is put into product production. The smaller the genome needed to perform a task, the less energy is needed and also less waste is produced.

Minimizations of genomes use gene knockout techniques that either undergoes the homologous repair pathway or the non-homologous neighbour end joining pathway. Some of these techniques, such as ODM and CRISPR/CAS, are currently in a regulatory limbo and may or may not be regulated. If the techniques fall outside of regulation, like the current status of ODM and CRISPR in the US, then also the product and cells produced by these techniques may fall outside of the regulation. This is a challenge, as a no-regulated status will prevent, through removing the incitement for biosafety research, advancement in analytical methodologies and the understanding of the limitations of the technology. There will be little or no attempts to investigate off target effects and under which conditions such effects are prone to occur.

Even though minimal cells most likely have a severely reduced potential for survival outside of a bioreactor, there are a number of concerns that should be taken into account when considering synthetic organisms created with genome minimization approaches. Even though the growth in a potential accidental release environment would be difficult, the organisms modified are organism optimized for enhanced growth rate. As such, there is a huge positive selection pressure for survival-mutations. Different receiving environments have different existing microbiomes and it is therefore difficult to generalize on the likelihood for negative impact through survival of, and/or horizontal gene transfer from, minimal cells. A lot of research is needed to unravel this potential for negative effects. More knowledge on how natural systems (i.e. endosymbionts) with minimal cells interact with the environment, could give us a much better understanding of factors important for a robust risk assessment.

4. Synthetic Genomes and protocells

The opposite of a top-down approach, are the bottom up approach, which would be to construct life/cells starting out with no DNA. Craig Venter and the artificial bacteria, Synthia, is the most known example of synthetic genome construction, but also protocells, which are vesicles without any cell wall, can be constructed to carry synthetic material. Building novel cells and new life, is, of course, an extremely useful tool to understand how life came about and what is needed to generate a living cell. These are novel, self-replicating living systems and as such, could have a long lasting environmental effect if released into the environment. Because there would be no prior experience on how the particular novel living life form would interact with the environment, the effects are largely unpredictable. As with minimal cells, the likelihood of survival outside of a bioreactor may be limited, but the cells may have unintended and unknown potential for adaptation and environmental interactions. The EU commission notes in the opinions, that synthetic genomes are yet far from commercial applications.

5. Xenobiology

Xenobiology, sometimes referred to as chemical synthetic biology, refers to the construction of life using non-canonical basepairs and amino acids. These components do not exist prior in nature and the construction of biological systems based on this principle must be considered as a completely new chemistry. Roughly we can divide the xenobiology approach into two categories,

1) Non-canonical DNA or amino acids that are recognized and used by already existing cell components like DNA and RNA polymerases, and
2) Components that are not intended to be recognized by natural systems at all. These components shows a great promise in biocontainment strategies (see later).

The use of xenobiology can be applied to research for understanding development of life, biocontainment and to new compounds used in pharmaceuticals and other biotechnology applications.

Molecules with changed sugar-backbone are called XNA, where X refers to any moiety (could be G for glycol or H for hexitol) and NA stands for nucleic acids. Changing the backbone of the DNA/RNA from ribose or deoxyriobose to something not seen before in nature, like glycol or hexitol (GNA or HNA) could preserve the normal binding capacity of canonical basepairing, but prevent DNA polymerases and RNA polymerases to read the information. Resistance to natural DNA degrading enzymes may be higher with XNA molecules and therefor XNA would potentially have increased

stability inside and outside of cells (i.e. persist longer in nature). New ways of storing information could potentially enlarge the number of basepairing possibilities and allow more information to be stored in shorter XNA-stretches. It is unclear whether or not the development within xenobiology and XNA molecules will give rise to new life forms, either bacteria or higher organisms.

Given that the new xenobiotic organisms are not yet developed, it is difficult to predict relevant biosafety concerns on the basis other than speculations. However, many concerns connected to GMOs and, in particular GMMOs (GMMOs), are also relevant for synthetic organisms created by xenobiolog. For instance, a careful description of the potential receiving environment is necessary to assess the synthetic organisms' evolutionary fitness and ecological competitiveness. In this, the novel organisms' susceptibility to virus, diseases and predation, play a major role. For instance, if the receiving environment have a low biomass, maybe due to pollution that needs to be remedied, the spread and survival of new organisms are easier (i.e. the ecological niche is not occupied). If xenobiotic organisms are not being put under the same evolutionary pressures as the existing community (i.e. they can live of the pollution), it is greater possibility that they can establish a sustainable population. Pauwels et al., 2012 lists a number of biosafety concerns that almost all are connected to preventing survival of the novel organisms or transfer of XNA. It is already a likely outcome that XNA may be harder to degrade than DNA. To avoid potential uptake of XNA and incorporation into genomes, it may be necessary to investigate the XNA in different cell systems and to take measure to avoid that it is recognized and transcribed by existing cell systems. This is not an easy task, as a recent publication has found that one XNA; Threose Nucleic Acid, can be transcribed into DNA by a naturally occurring DNA polymerase from *Geobacillus stearothermophilus* (Dunn and Chaput, 2016). Given the diversity in nature, there is no reason to think that this is a unique example of cross-reactions between XNA and natural proteins. Potential uptake and incorporation of XNA or XNA derived DNA into microbial genomes, are therefore an example on possible unwanted outcomes of interactions between synthetic nucleic acids and natural ecosystems.

Xenobiology in biocontainment strategies

To minimize the risk of unintentional release, the introduction of biological safety locks in biocontainment strategies. One big challenge with biological safety locks today are that they are not absolutely fool proof because many factors like, mutations combined with high positive selection for mutants and the possibility for cross feeding on other metabolites, weakens the biocontainment. Supplying bacteria that need non-canonical chemistry to multiply and survive, with the needed synthetic chemicals (XNA) is a possible improvement of current biocontainment strategies and if properly implemented, will give an extra layer of safety. Challenges like bacterial fitness must of course be overcome and still a lot of research remains to reach a goal of a better genetic firewall (Acevedo-Rocha and Budisa, 2016). From a biosafety perspective, the degree of possible horizontal gene transfer and back mutations must be minimized for a safe product. The engineered microbe must not be able to survive in any natural habitat. In addition, the natural enzymes must not be able to function on the non-canonical or orthological XNA molecules, like the polymerase from *Geobacillus* has been shown to do. It is a significant challenge to safely implement these conditions given the biodiversity and the level of undescribed species that exist among soil and water bacteria.

6. Supporting technologies – CRISPR/Cas

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a bacterial immune system that stores the history of viral infections in the bacteria. This memory bank makes the bacteria able to rapidly respond to a new infection by a virus it has seen before and helping the cell to overcome the infection. Because the memory system and bacterial immune system works on the DNA level, this immune system can be manipulated to target many DNA sequences from different species and mark this DNA sequences for degradation by the bacterial Cas protein. The use of CRISPR/Cas as a tool in synthetic Biology and genetic engineering has exploded the last two years. Genome-editing, like by the use of CRISPR/Cas, differs from classic genetic engineering techniques because it opens up the opportunity for target modification, i.e. the modification of specific regions and sequences in the genome. Several technologies sort under genome editing techniques, i.e. Zinc-finger nucleases and oligo-directed mutagenesis, and all of these are possible to use as supporting technologies in synthetic biology. This is particular true for the top down approach (produce a minimal genome) where genome editing techniques can be used to stimulate targeted genome deletions. With particular CRISPR/Cas now being cheap, efficient, accessible and flexible, the arrival speed of available applications is of great concern because the development of regulations or biosafety relevant research are seriously lagging behind. Particular the possibility for multiplexing with several RNA molecules (CRISPR) utilizing the same Cas proteins and thereby introducing many changes at the same time in the genome, has implications for regulation and safety tests. Normally biosafety tests are done on a basis of comparisons, and with huge changes in the genome, there are no comparator that are suitable. In a GMO with many insertions, for instance, each insertion is generally assessed on its own, but with many changes introduced at the same time, gene edited synthetic biology organisms must be assessed with all the changes introduced. This creates a possibility for synergistic effects and in combination with no comparator, it is very difficult to predict unintended effects in the synthetic organisms.

7. General biosafety concerns of synthetic biology and organisms/products thereof

Monitoring of sites with contained and open use of fermentors may be required with the purpose to survey unintended releases, escapes and accidents. Because most of the synthetic organisms today are microorganisms, detection of a new population though escape, is very difficult. Factors like lag time before a population is established and high detection limit in soil/water, hampers effective surveillance. Gene transfers are even more difficult to survey and once transferred, the DNA or XNA could persist at a low level in the population.

The bacteria used in synthetic biology, generally have a high potential for rapid change. This is because the degree of changes imposed on them through genetic manipulation must be endured. Because of this feature, such bacteria may have a higher potential to adapt again to a natural environment, and pose a biosafety concern or even a hazard.

Even though many synthetic organisms would face huge obstacles to overcome to survive in nature, huge quantity of cells released will expose huge quantities of DNA/XNA, maybe with high persistence due to non-canonical backbones in the DNA/XNA, to the environment. Unintended release of bacteria from bioreactors are more likely to occur in huge quantities than low quantities and algae that escapes biocontainment could bloom and generate huge amount of DNA/XNA. We need to

understand, on a case-by-case basis, the survival possibility of the synthetic organisms in question and the persistence of that specific DNA/XNA in the environment. This must be coupled with a better understanding of horizontal gene transfer (HGT) mechanisms and potential of spreading the XNA in a population. It is important to understand that HGT also include uptake of DNA into the synthetic organisms, subsequently such uptake may have a potential for inactivating some genetic firewalls, with the consequence that biocontainment may fail.

Synthetic biology is not a new form of science, but the new advancement particular in gene knockout and genome editing methods, has caused many new applications to be developed in a short time. Because the applications in animal breeding and crop development are relatively new, biosafety related questions are plentiful. For a technology like CRISPR/Cas, there are lack of studies that investigates unintended effects, off target effects and the effects of pathway engineering. One of the primary arguments for doing such research are that even though the introduced change may be small, the effect may be much higher in magnitude particularly if one generate a knockout of a pathway or changes protein affinities (enzyme-substrate interactions). Disease resistance in animals and plants are examples of virus-targets knockouts (i.e. porcine reproductive and respiratory disease virus resistant pigs- CD163 knockouts). The full debate of regulation or not of CRISPR/Cas edited genomes are not within the scope of this report, nevertheless it seems clear that without regulations put in place, the incitement for biosafety research stands the risk of being weakened.

Synthetic biology is not primarily about gene editing or gene knockouts, but about combining different areas of science into a new way of thinking. Societies like igem (http://igem.org/Main_Page), Do-It-Yourself biology (http://diybio.org) and biohackers, promotes the idea of solving real world problems by building genetically engineered biological systems. It may be a concern that the machine-like approach of modular genetic design, weakens the understanding of biological systems and thereby the critical thinking connected to genetic engineering (i.e. consequences, unintended effects, impact on environment and health etc.). Even in applications that uses DNA for computer transfer and storage, must take into consideration that active biological parts may be a source for environmental impact. Public engagement and science communication in synthetic biology and in biosafety may be a tool that can help to promote the understanding of safe application of synthetic biology. The SynBio dialogue was a project initiated in 2007 and finished in 2010 (http://www.bbsrc.ac.uk/engagement/dialogue/activities/synthetic-biology/). It promoted the synthetic biology views, concerns and aspirations of UK citizens, and aspires to allow these views to influence future policies. Now, in an area with new applications and rapid technology development and a large degree of debate and disagreement of safety within the scientific community, a broad public engagement could help facilitate the outcome of this debate. One of the conclusions from 2010, is interestingly that people find synthetic biology both exiting and scary. To overcome public scepticism and to strengthen the social responsibility of scientists, allowing a broad discussion on both risks and opportunities and considering a variety of perspectives, coupled with an effective dialogue between stakeholders, should lead to more sustainable decisions and improved public acceptance of the use of synthetic biology and products produced thereof. (http://www.bbsrc.ac.uk/documents/1507-synthetic-biology-deliberation-aid-pdf).

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Risk assessment of SynBio organisms and products: Lessons from GMOs

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While there is no internationally agreed definition of "synthetic biology", key discussions on the potential positive and negative impacts of synbio organisms and products have been held within the GMO community. Not surprisingly, many of the supporting techniques applied in synthetic biology are also used in classic genetic engineering (e.g. agrobacterium-based genetic transformation, biolistic, plasmid construction, molecular cloning, DNA amplification and ligation, etc.). Therefore, discussions on potential impacts of synthetic biology can be built on the lessons learned from many years on risk assessing modern biotechnology-derived organisms and products (SCBD, 2015).

The Convention on Biological Diversity, the Cartagena Protocol and its Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress are the international treaties addressing threats of significant reduction or loss of biological diversity posed by organisms, components and products resulting from modern biotechnologies, including GMOs. The Conference and its protocol have a clear statement urging its Parties and inviting other Governments to take a precautionary approach, in accordance with the preamble and with Article 14 of the Convention. Under the Convention, it is recognized "where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat" (CBD, 1992). Although not all regulatory frameworks include the precautionary approach, the principle has been much used by regulators, scientists, civil society organizations and others when assessing new and emerging technologies that lack substantial knowledge on their potential risks. Much of what will be discussed in the following paragraphs regarding the molecular characterization step of pre-market risk assessment of synbio organisms and products will take into consideration the precautionary approach.

Molecular characterization refers to the description and identification of all genetic modifications and changes performed on the host organism to produce a synthetic biology organism and/or product. Most importantly, the molecular characterization step of the pre-market risk assessment should emphasize the identification of hazard. Its relevance to risk assessment is related to the introduction of potential risk pathways created by synthetic biology that can be evaluated and mitigated through the use of molecular biology techniques (e.g. DNA amplification and PCR, DNA sequencing, protein quantification, etc.). It is also relevant to consider novelty, complexity and exposure of such new organisms and products. The molecular characterization that is relevant to risk assessment includes, among others: DNA and RNA sequences, transcription (how RNA is made), post-transcription (how RNA is processed), translation (how protein is made), post-translation (how protein is changed), enzyme-mediated chemical reactions (metabolic pathways), etc.

The most common approach to the identification of hazard in this step is by a comparative approach through the use of an appropriate comparator. Appropriate comparators are the non-transgenic parental line or lines with the nearest genetic material as possible. EFSA has several guidelines on

how to choose the best comparator (EFSA, 2011a). The identification of changes between the synthetic biology organism and the suitable comparator should not be interpreted as hazard per se but instead, they should help to identify potential differences, which will then be subject to further toxicological investigation.

Over these years, we have learned from GMO molecular characterization a variety of intended and unintended changes. Intended effects are those targeted to occur from the introduction of the gene(s) in question and which fulfils the original objectives of the genetic modification process. On the other hand, unintended effects are considered to be consistent differences between the GM plant and its appropriate control lines, which go beyond the primary expected effects(s) of introducing the target gene(s). Unintended effect(s) could potentially be linked to genetic rearrangements or metabolic perturbations. They may be evident in the phenotype or composition of the GM plant when grown under the same conditions as the controls (EFSA, 2011b). Some of the well-known unintended effects observed in GMO commercialized varieties includes the lack of terminator sequence in MON810 maize event (OECD unique identifier MON-ØØ81Ø-6) (Rosati et al., 2008), which was identified by independent scientists almost a decade after its first introduction in the US. These researchers have also found the production of a series of fragments with different lengths suggesting the synthesis of RNA variants (Rosati et al., 2008). The results of that study were never properly followed through but suggest to be related to the different toxicological effects observed in off-target species not suppose to be susceptible to Bt/CRY proteins (Hilbeck and Schmidt, 2006). Another example is the presence of a 254bp portion adjacent to the 3'end terminator sequence of truncated CP4EPSPS protein followed by a DNA segment of 534bp of plant origin in Roundup Ready soybean (OECD Unique Identifier MON-04032-6) (Windels et al., 2001). Further studies were perform to examine the functional importance of such additional DNA fragments and read-through product was detected, resulting in four different RNA variants from which the transcribed region of the terminator is completely deleted (Rang et al., 2005). These transgene products variation might explain why Bt protein content in transgenic plant varieties has been highly contested and also stated by many scientists to be difficult to be predicted, especially in plants grown in stressful conditions (Trtikova et al., 2015). Unintended effects are, therefore, a legitimate concern due to the potential genetic rearrangements or metabolic perturbations from transgene insertion.

The potential lack of suitable comparators in synthetic biology has been noted by some members of the Ad Hoc Technical Expert Group (AHTEG) On Synthetic Biology under the CBD (CBD, 2015). When discussing the degree to which the existing arrangements constitute a comprehensive framework in order to address impacts of organisms, components and products resulting from synthetic biology, in particular threats of significant reduction or loss of biological diversity; members have noted that that current risk assessment approaches and methodologies must have to be adapted to address matters that are of particular relevance to synthetic biology. They have also identified the "lack of familiarity in comparison with non-modified organisms, challenges in establishing meaningful comparators, and possibly higher levels of uncertainty as gaps in the existing methodologies for assessing the environmental impacts of organisms of synthetic biology, and identified a need for guidelines and capacity-building to be developed and made available" (CBD, 2015). Recently, the Subsidiary Body On Scientific, Technical And Technological Advice has also acknowledged the same understanding on their recommendations document (SBSTTA, 2016).

In fact, synthetic biology may transfer "whole systems," rather than single traits and few transgenes as in commercial GMOs. Therefore, due to its complexity, it is insufficient to rely on risk assessments based on individual trait or parts. This is because the interactions among the parts have no comparable counterpart in nature, making it more difficult to predict the cell's full behavioral range with a high degree of certainty (Schmidt, 2009). Adaptations to current risk assessment methods will then have to address the potential lack of an analog in the natural world as a comparator as well as the combinatorial effects of new genetic material and new traits.

While some national and international treaties include frameworks for risk assessment, sufficient information may not be available for all synthetic biology techniques to effectively conduct risk assessments. There is also no agreement among scientists, industry and civil society, how well the potential dangers related to synthetic biology are known and how they can be assessed (CBD, 2015). The potential lack of a suitable comparator in GMO analysis has been pointed by some scientists and EFSA in previous documents not related to synthetic biology. These authors suggested that in situations where a suitable comparator, the whole GMO is considered a novel genotype in the receiving environment. On a case-by-case basis, information available from "omics" technologies, as it becomes available, may help to detect phenotypic and compositional changes that cannot be detected using a comparative approach (Heinemann et al., 2011; EFSA 2011a; AHTEG, 2010).

"Omics" technologies refer to a field of study in biology in which high throughput analyses are applied to generate large-scale data-rich biology. Classic examples are genomics, transcriptomics, proteomics and metabolomics. These are untargeted profiling approaches that usually comprehend quantitative surveys of broad classes of molecules (Heinemann et al., 2011). In fact, the use of "omics" analyses is an evolving field and a recent review on the applicability and usefulness of molecular profiling techniques for GMO risk assessment is available in Heinemann et al. (2011). These authors concluded that a broader use of molecular profiling in a risk assessment may be indeed required to supplement the comparative approach to risk assessment of new and more complex GMOs. In addition, they highlighted that the literature-based discussions on the use of profiling appear to have settled that profiling techniques are reliable and relevant, at least no less so than other techniques used in risk assessment. Although not required routinely, the dismissal of routine molecular profiling may be confusing to regulators who then lack guidance on when molecular profiling might be worthwhile. We consider "omics" technologies or molecular profiling an important way to increase confidence in risk assessments for any GMOs but also to new synthetic biology organisms and products. If the profiles are properly designed to address relevant risks and are applied at the correct stage of the assessment (Heinemann et al., 2011).

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Adequacy of current methodologies for environmental risk assessment of synthetic biology

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The emergency of new biotechnologies is frequent and has happened many times during the past decades. Synthetic biology as an evolving field of genetic engineering is just another example on how technologies develop within society with aim at providing beneficial outcomes. The question still remains on how to contribute to the safe use of new biotechnological applications? We are unlikely to know and understand everything about every organism. And so, we should agree on some convenient way to study these new organisms in great detail and use experience from the past to build on in the future. International and national regulations have developed a framework for a pre-market risk assessment of genetically modified organisms and we can definitely build from that.

There are several foreseen applications of synthetic biology. Applications to replace natural materials (e.g. squalene, vanillin, etc.); bioenergy; biosensors; applications to alter wild life populations (e.g. adapting coral to survive higher temperatures, gene-drives, de-extinction of certain species, etc.); applications for chemical production (e.g. bio-plastics) and agricultural applications (e.g. crops to tolerate climate change, crops to produce new pesticide or fertilizers) (SCBD, 2015). The range of synthetic biology applications exemplifies the different types and characteristics of such synthetic biology organisms and products. While some might present less complexity and novelty compared to current commercial GMOs, some might represent a completely new organism. Therefore, prospect on the adequacy of current methodologies for environmental risk assessment of synbio products might depend on how the novelty and complexity is perceived.

A recent debate over a new type of GMO, so called RNAi-based GM plants, had much to teach us about why and how to adapt risk assessment frameworks to new kinds of GMOs. While some regulators considered RNAi-based GM plants no different from any other GM plant (Heinemann et al., 2013), others have acknowledged that such new type of GMO might affect their present approach for risk assessment (EFSA, 2014). The European Food Safety Authority (EFSA) has organized an international scientific workshop in June 2014 to discuss potential risks associated with ribonucleic acid interference (RNAi)-based genetically modified (GM) plants and to identify issues unique to their risk assessment. During the workshop, the molecular biology underlying the RNAi mechanism, current and future applications of RNAi-based GM plants, and risk assessment aspects were discussed in details. 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). Later that year, EFSA called a Tender to obtain a comprehensive literature overview on several of the risk assessment-related issues identified during the EFSA's workshop and the outcome of this systematic literature search/literature overview will support the risk assessment considerations for RNAi- based GM plants (https://www.efsa.europa.eu/en/tenders/tender/ocefsagmo201501). In the meantime, Brazil, New Zealand and Australia have approved RNAi-based GM plant events for environmental and food/feed commercialization without any changes or adaptations in their risk assessment procedure. In fact,

these regulators used a priori assumptions that they did not need to do a risk assessment of novel dsRNA molecules, rather than requiring experimental evidence that these molecules caused no adverse effects (Heinemann et al., 2013). This is a clear example on how different regulators perceive the novelty and how they decide to act.

Similar debate on the adequacy of existing national, regional and/or international instruments to regulate the organisms, components or products derived from synthetic biology techniques has also happened under the CBD umbrella. The Open-ended Online Forum on Synthetic Biology was established in decision XII/24 and started its activities on April 2015. The objective of the Online Forum was to support the work of the Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology by providing information that is relevant to its mandate (http://bch.cbd.int/synbio/open-ended/discussion.shtml). The outcomes of these discussions were complied in a report document made by the AHTEG on Synthetic Biology, which is also available online (AHTEG, 2015). The document reports that several submissions and online interventions noted that most of the current commercial and near-commercial applications labeled as synthetic biology would be considered a GMO developed through modern biotechnology due to genetic modification of these organisms and the insertion of DNA sequences. Consequently, living organisms generated through synthetic biology would then fall within the scope of the Convention and its Protocols, as well as under existing national biosafety frameworks (AHTEG, 2015). On the other hand, there is a potential "grey area" in which some research areas of synthetic biology (e.g. gene editing, protocells and orthogonal systems) could raise potential issues with regard to their regulatory status as they may or may not be considered LMOs as per the definition in the Cartagena Protocol. In such cases, the majority of the Parties and other relevant organizations have pointed to the need to expand the language of the Protocol with a view to include such cases (AHTEG, 2015).

However, if in one hand, Parties of the CBD mostly agree that synbio organisms and products will fall within CBD and its Protocols, that doesn't mean that risk assessment frameworks contained in such treaties are necessarily adequate to address environmental, health, and societal concerns posed by organisms and products of synthetic biology. This reasoning is also reflected in the submissions to CBD, which pointed to the need for regular intervals revision on risk assessment frameworks to account for the rapid progresses in the approaches and techniques of synthetic biology. The degree to which the existing arrangements constitute a comprehensive framework in order to address the impacts of synthetic biology has also had a dedicated topic within these discussions. Although there was a certain level of agreement among the submissions and online interventions that the principles and methodologies of risk assessment, as well as risk management measures, established for LMOs can serve as a basis for addressing potential adverse effects associated with organisms developed through synthetic biology; others noticed that in the future, synthetic biology is likely to lead to the development of organisms that will differ fundamentally from naturally occurring ones, which will raise specific challenges and limitations and in such cases, risk assessment methodologies will need to be revised and adapted (AHTEG, 2015).

Two major concerns regarding the adaptation of current risk assessment frameworks have been highlighted by these expert groups under the CBD (AHTEG, 2015):

i) Lack of suitable comparators, and

ii) Impacts of organisms with intentional introduction into the environment and which are capable of replicating or reproducing, which risk assessors have minimal experience.

The comparative risk assessment system based on the familiarity principle and case-to-case approach will be challenged as new synbio organisms lack familiarity to natural organisms. Engelhard et al. (2016) have suggested special importance for an ongoing dialog between scientists and society in order to prevent acting under uncertainty with respect to risks to humans and biodiversity in the cases of low familiarity to natural organisms, as well as the implementation of the precautionary principle.

The "familiarity" concept in the GMO risk assessment is no different from the comparative approach to similar organisms proposed in the "substantial equivalence" term in OECD document back in 1993 (OECD, 1993) and applied by many regulatory agencies to date. Because of the comparative nature of the substantial equivalence approach, only effects of the genetic modification should be assessed. In fact, the major innovation needed in risk assessment for synthetic biology will be that the substantial equivalence approach will have to be modified and finally even abandoned, because the newly designed organisms will be intentionally more and more alienated from the genome of existing organisms (Engelhard et al., 2016).

Substantial equivalence approach is much based on targeted profiling approaches that usually refers to pre-determined assessment endpoints on the genome, proteome and metabolome levels (e.g. compositional analysis, southern or western blot analysis, etc) of a case to establish the similarity of GMOs and conventional counterparts (Heinemann et al., 2011). On the other hand, future non-comparative approaches will have to provide information to fill the gap in the hazard identification step of a risk assessment. In these cases and other cases, it has been propose that profiling, or molecular phenotyping, which monitors a population of RNAs, proteins, metabolites or their dynamic interactions, in a cell or organism at a particular timepoint and under defined conditions, will be the best candidate (Heinemann et al., 2011). Such profiling approaches are untargeted and comprehend quantitative surveys of broad classes of molecules without prior knowledge of them.

In summary, the methodology that is currently in use to assess the risks of GMOs can indeed provide a basis for the risk assessment of living organisms developed through synthetic biology. However, there is a need to continue to revise and further develop risk assessment methodologies in order to fully address the potential environmental and societal impacts of future synthetic biology applications. The approach of whole organisms analysis using untargeted "omics" techniques has been pointed as one possible element of a way forward.

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Ensuring Synthetic Biology Safety: lessons from the era of Virus-based GM Vaccines and Gene Therapy medicinal products

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Genetically modified gene therapy medicinal products and GM vaccines

Genetically modified (GM) gene therapy medicinal products (GTMP) generally consist of modified somatic cells, which are administered to human or animal subjects with a view to correcting, restoring or modifying physiological functions in these subjects. Viruses constitute greater than 70% of the vectors used in GM-GTMP (Gene Medicine, 2015). Viral vectors can be replication deficient or replication competent, and are genetically modified usually to express specific proteins. By using these vectors genetic modification of somatic cells can be achieved.

GM virus vaccines use virus vectors as gene carriers to induce immune responses against foreign (transgenic) antigens or against virus particles in which certain genes have been modified by techniques of recombinant gene technology. Diseases that are target of GM vaccines are those against which achieving therapeutic or prophylactic protection is currently difficult or impossible. Examples include tuberculosis, Ebola, HIV, malaria (human diseases); and rabies, canine distemper, rinderpest (veterinary diseases). Viruses commonly used as vectors in GTMP and GM vaccines are retro viruses, poxviruses, adeno viruses, adeno-associated viruses, lentivirus, and herpes simplex virus. This chapter focuses on GM vaccines and GTMP in which the vector employed in the modification is a virus (as against other vectors such as bacteria, bacterial plasmid DNA, and approaches designed to modify or inhibit the functioning of an endogenous gene or genetic elements in mammalian cells).

Some virus-based genetically modified gene therapy medicinal products and GM vaccines are also synthetic biology products

Current strategies in the design and production of GM-GTMP and GM vaccines are aimed at increasing their potencies. Consequently, these strategies are witnessing accelerated advancement in genetic modification technologies, some of which are synthetic biology techniques. Today, there are several definitions of synthetic biology (Convention of Biological Diversity, 2015). For risk assessment purpose under the European Union (EU) framework, the most recent and acceptable definition of synthetic biology is the definition adopted by the European Commission: “the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms” (Breitling et al. 2015). Although there is no consensus definition of synthetic biology or a common agreement of the techniques that can be grouped under this technology, products of synthetic biology are generally perceived as those which consist of or contain biological components or possess properties which are not presently found in nature.

Common examples of synthetic components currently used in viral vectors of GM vaccines and GTMP include:

i) A GM virus vaccine (recombinant modified vaccinia virus Ankara (MVA)) that uses a synthetic promoter for the expression of two genes (haemagglutinin and nucleoprotein) of the influenza virus (Sutter et al. 1994). The synthetic promoter is a stronger driver of gene expression relative to the natural promoter of the virus, thus, permitting a high expression of the two foreign genes. This provides enough gene products, haemagglutinin and nucleoprotein, to stimulate protective immunity against the influenza virus.

ii) A gene therapy virus vector, Delta-24-RGD, which is an adenovirus oncolytic vector modified by the insertion of a synthetic RGD motif (comprising of synthetic Arginine-Glycine-Asparagine) in a protein component (fiber protein's HI loop) of the vector's epitope responsible for binding and infection (Jiang and Fueyo 2010). This modification allows the virus to enter glioma (brain tumor) cells by the integrin receptor not normally used by the virus, because the virus's native receptor CAR (Coxsackie virus and adenovirus receptor) expression in glioma cells are relatively low. Thus, the modification using the RGD synthetic motif enhanced tropism by retargeting the vector to a cell type (glioma) which naturally is refractory to adenovirus infection.

What are the implications of synthetic biology approaches in ERA?

In the traditional approach to genetic modification, constructs are designed based on gene of interest identified in a donor organism, and inserted and expressed in a recipient organism. The environmental risk assessment (ERA) of a synthetic approach will not be different from the ERA of a traditional genetic modification if the synthetic sequences are exact copies of naturally existing sequences, because the ERA will be based on the known functions of the latter. Notable examples include:

1) Combination of sequences from different sources where the traditional cloning approach cannot be applied, such as the combination of a protein coding sequence and a regulatory sequence, or chimeric genes in which parts of different genes are joined together to produce a gene that encodes a protein whose function is distinct from the functions of the original genes.
2) The replacement of a natural promoter by a synthetic construct, where the binding motif in the synthetic construct has been modified for greater affinity with polymerases.
3) Introduction of changes in the codon of protein coding sequences during DNA synthesis in order to adjust the codon use in the recipient organisms in which the DNA will be expressed.
4) Introduction of point mutation to change the properties (e.g. specificity and activity) of the encoded protein.
5) Addition of domain coding sequences which introduces new function(s) in a protein.

However, the rational design from scratch of novel gene sequences with no existing architype in nature, e.g. design of DNA sequences whose protein functions are predicted using bioinformatics, may confound its ERA. Rational design of novel gene sequences is a more recent synthetic biology approach to GM vaccines and GTMP, and currently has fewer examples. An example is the gene shuffling of human papillomavirus proteins E6 and E7, and fusing same to tetanus toxin fragment C domain 1 (Henken et al. 2012). Gene shuffling was performed to avoid the risk of inducing malignant

transformation at the vaccination site. Thus, the shuffled vaccines lose their oncogenic properties but retain immunogenicity (Henken et al. 2012).

What is the current procedure for risk assessment of GM vaccines and GTMP comprising of or consisting of synthetic components under the European Commission?

The ERA is fundamentally based on comparison between the modified organism and its closest relative with the aim of identifying differences that may constitute hazard to non-target organism because of the modification. Thus, knowledge of the function of the gene in the donor organism is crucial to deducing the changes that genetic modification may impact on the recipient organism. Currently in the EU, there is no separate procedure for the ERA of synthetic GM vaccines and GTMP. The European commission (EC) opinion, published in January 2015 (Breitling et al. 2015), did not recommend a separate approach in the risk assessments of products containing or comprising synthetic materials or products generated using synthetic biology approaches. This opinion is hinged on the EC's rather broad definition of synthetic biology: "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms" (Breitling et al. 2015). The EC further emphasized that synthetic biology and genetic modification are fundamentally the same albeit continuously evolving field; and that existing regulations and guidelines for biological and genetically modified materials apply to synthetic biology materials (Breitling et al. 2015). However, there are several other different definitions of synthetic biology (Convention on Biological Diversity 2015). Although the definitions are different, they generally provide the perception that synthetic biology is the design/construction of biological components and/or systems in a way in which the systems and/or biological components do not exist in nature. Lack of a consensus operational definition of synthetic biology may impact ERA of GM vaccines and GTMP, because an operational definition is required to formulate ERA risk assessment framework.

Will synthetic biology approaches overburden current environmental risk assessment procedures?

The recommendation of the EC for the continued use of ERA existing framework for synthetic biology product raises the question: will synthetic biology approaches overburden the current ERA procedures of GM vaccines and GTMP? For synthetic GM vaccines or GTMP products that have no existing prototype in nature, the challenge will be to find suitable isogenic comparator. A suitable natural isogenic comparator is required for a GM viral vector because properties of the vector or its adverse effects can be predicted on existing familiarity with the wild type virus and with the effect that the insert may have, based on the known roles of the inserted sequences in the isogenic or wild type donor organism.

The first part of an ERA is the identification of properties of the GMO, on a case-by-case basis, that can constitute hazards to the environment. Here, we attempt to outline some common risk factors for the environment, bearing in mind that only the surrounding environment, including the living ecosystem (and not the target individual for application of the GM product) is considered. In the EU, a standard ERA of GM medicinal products addresses the following main areas: risk to humans, risk to

the environment and subsequently assessment of the overall risk (European Medical Agency 2004, European Commission 2006). The environment is defined as the surrounding ecosystem including animals, plants and microorganisms.

The following main points are considered in ERA:

Hazard identification: This includes hazardous characteristics of the GM vaccines and GTMP that could lead to harm to the environment. This concerns the capacity of GM vaccines or GTMP to transmit to non-target species, shedding of live viruses into the environment, capacity to survive, establish and disseminate, pathogenicity to non-target organisms/individuals, potential for gene transfer, nature and properties of inserted sequences including phenotypic and genotypic stability.

Assessment of likelihood: Encompasses the probability and frequency of the identified hazards.

Assessment of the level of risk: Involves the estimation of the combined effects of the above components of hazard and its subsequent likelihood of occurrence. Here, a risk matrix can be employed to illustrate the estimation (see table 1).

Table 1: A risk matrix to illustrate risk estimation

		Likelihood of hazard			
Magnitude of hazard		**High**	**Moderate**	**Low**	**Negligible**
	Severe	High	High	Moderate	Negligible
	Moderate	High	High	Moderate/Low	Negligible
	Low	Moderate/Low	Low	Low	Negligible
	Negligible	Negligible	Negligible	Negligible	Negligible

Adapted from Guidelines for Applicants (European Medical Agency 2005)

Assessment of the consequence: Similar to estimation of risk, evaluation of the consequence in the event of an adverse effect can be estimated by employing the risk matrix. Each potential consequence is assigned a relative weighting on the standards of high, moderate, low or negligible (see table 2).

Table 2: A risk matrix for estimation of consequence.

		Likelihood of hazard			
Consequences of hazard		**High**	**Moderate**	**Low**	**Negligible**
	Severe	High	High	Moderate	Negligible
	Moderate	High	High	Moderate/Low	Negligible
	Low	Moderate/Low	Low	Low	Negligible
	Negligible	Negligible	Negligible	Negligible	Negligible

Adapted from Guidelines for Applicants (European Medical Agency 2005)

Assessment of the overall risk to the environment: A weight of evidence approach is usually employed because estimates are often qualitative.

Risk management strategy: In the final step of the ERA of GM vaccines and GTMP, when the overall risk to the environment has been determined, it is necessary to evaluate whether risk management

strategies need to be implemented in order to minimize the occurrence of the identified potential hazards. A set of relevant protective measures has to be proposed in cases where the overall risk to the environment is not negligible. However, the basic approach to minimizing risk is best addressed during product design and development.

What are the gaps in ERA procedure that may be challenging if applied to synthetic GM vaccines and GTMP products?

A major paradigm in the ERA of GM vaccines and GTMPs is that properties of the vector and inserts, as well as their potential adverse effects due to the modification can be predicted based on existing familiarity with the wild type virus and the functions of the insert in the original donor organism. Therefore, the ERA of synthetic GM vaccines and GTMPs with synthetic sequences based on existing natural prototypes would be easily guided by the foreknowledge of the original parents/sources of inserts. In such cases, areas of focus would be the examination of protein products of synthetic sequences for changes in their amino acids. If changes are identified that have no examples in nature, there will be gaps in the so-called foreknowledge of the original proteins, which are necessary in order to establish their safety, even though their sequences were based on naturally occurring prototypes. This gap in knowledge is located in:

(i) The lack of certainty of the correctness of the prediction of their functions;

(ii) The possibility that the changes in amino acids may confer an unknown and unexpected function to the protein, which may have adverse effect.

In the case where part of a GM vaccine or GTMP is based on *de novo* synthesis using e.g. bioinformatics approach where the new protein does not have a prototype in nature, it becomes difficult to find appropriate parental comparator to guide risk assessment questions and experiments.

Conclusion

The era of GM vaccines and GTMP can be said to have started in the 80s with the first documented unsuccessful attempt at gene therapy for β-thalassemia in 1980, and the production of recombinant rabies vaccine in 1984. Therefore, important lessons can be learnt from the experience that can help guide how to evaluate the ERA of synthetic virus based vaccines and GTMP. Currently, what constitute synthetically modified vaccines and GTMP are synthetic vaccines or GTMP in which part of the vector sequences are synthetic sequences. The ERA approach to these products currently largely depends on whether there exist a prototype in nature to which they can be compared. Comparison to an original parent or source of a transgene is fundamental in ERA because it helps to deduce whether the applied modification has resulted in hazardous changes in the new organism that may be deleterious to the environment. At present, there is no consensus operational definition of synthetic biology and this may confound the ERA of synthetic vaccines and GTMP. The European commissions has recommended the use of the ERA procedure of GMO for synthetic organisms, but there are existing gaps in knowledge in the ERA of GMOs that can confound its use for synthetic virus-based vaccines and GTMP.

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Socio-economic considerations with the synthetic biology-based products and applications

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Synthetic biology-based products and applications have started to enter the market and will be commercially available in a diversity of fields ranging from life sciences to biofuels. Besides assessment of risk there has been an increasing interest for the inclusion of broader assessments by the use and introduction of new technologies. For example, socio-economic considerations have been widely discussed with the use and introduction of genetically modified organisms (GMOs), and such considerations have been included in international regulative frameworks such as the Cartagena Protocol and national biosafety regulations. Due to the novelty of synthetic biology there is a lack of empirical studies of socio-economic impacts by its products and applications. Therefore, I will here draw on experiences with GMOs. This analogy is also relevant since many synthetic biology-based products and applications will fall under the GMO definition and hence be assessed under GMO regulative framework.

Socio-economic considerations in regulative frameworks

Socio-economic impacts have especially been discussed for the introduction and use of genetically modified (GM) crops. Their importance is reflected in increasing efforts to include their assessment in GMO regulatory frameworks as the Cartagena Protocol on Biosafety, European and African fora. By 2015, more than 34 countries have included socio-economic provisions in their GMO biosafety legislation (Binimelis and Myhr, 2016). In Europe, for instance, a new Directive on GMOs was approved in March 2015 (Directive EU 2015/412), allowing a Member State to adopt measures restricting or prohibiting the cultivation of a GMO or of a group of GMOs defined by crop or trait. Legal grounds for applying such measures include socio-economic impacts, avoidance of GMO presence in other products, agricultural policy objectives or public policy (Directive EU 2015/412, Article 1.3). At the international level the Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) states in Article 26.1:

> "*The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities.*"

Socio-economic considerations can also be found in the operational objective 1.7 of the Strategic Plan of the Cartagena Protocol on Biosafety for the period 2011–2020 where its aim is: "*To, on the basis of research and information exchange, provide relevant guidance on socio-economic considerations that may be taken into account in reaching decisions on the import of living modified organisms*". As a response to this objective, the Secretariat of the CBD launched several on-line discussions and appointed an Ad Hoc Technical Expert Group (AHTEG-Sec). One of the tasks by this group will be to develop an outline for guidance on the implementation of socio-economic considerations in biosafety decision-making (CBD, 2014a). The CBD Secretariat has also compiled

several reports (CBD, 2014b), as well as national surveys in order to assess how socio-economic considerations can be taken into account (CBD, 2014c). The importance of socio-economic considerations by synthetic biology-based products and applications has also been recognized by the Ad Hoc Technical Expert Group on Synthetic Biology (AHTEG-Synbio) (2015).

The AHTEG-Synbio (2015) emphased the need for further guidance for investigating and evaluating appropriate methods for integrating socio-economic considerations in the regulatory framework: *The assessment of the potential benefits and potential adverse effects of synthetic biology is therefore challenged by the difficulty of distinguishing which socioeconomic changes result from the introduction of synthetic biology. Under such circumstances, it may be necessary to introduce appropriate methods from relevant scientific disciplines to take socioeconomic considerations into account*.

The regulative frameworks established in GMO legislations have chosen different options in their scope and substantive requirements, as well as in their degree of implementation of socio-economic considerations (Binimelis and Myhr, 2016; CBD, 2014c; Falck-Zepeda, 2009; Spök, 2010). The EU Directive is unclear on what specific socio-economic impacts can be taken into account, as well on how to do it. In addition, neither the Cartagena Protocol or the EU directive describes what type of evidence and methodologies that are necessary to claim such a consideration or impact. This may be due to the lack of definition of what socio-economic are in the regulatory framework, thus of what is covered and what excluded. As a response to the lack of definition the AHTEG-Sec of the Convention of Biological Diversity, considered that the scope of the term includes five dimensions: (a) economic; (b) social; (c) ecological; (d) cultural/traditional/religious/ethical; and (e) human-health related (AHTEG-Sec, 2014).

The Norwegian Gene Technology Act

Norway was the first country to include broader issues in its GMO regulatory framework. The Norwegian Gene Technology Act of 1993 regulates the production and use of GMOs. For a GMO to be approved in Norway, the Act requires that the production and use of GMOs take place in an ethically justifiable and socially acceptable manner, in accordance with the principle of sustainable development and without adverse effects on human and animal health and the environment. Hence Norway is one of the countries with most experience in carrying out broader risk assessments.

In 2000, the Norwegian Biotechnology Advisory Board initiated a work on how to operationalise the concepts of sustainable development, social benefit and ethical and social considerations. This work was partially included in 2005 in the appendix of the Regulations on Impact Assessment pursuant to the Gene Technology Act. In Norway the socio-economic considerations include both potential beneficial and risk factors. According to Rosendal (2008), the inclusion of impacts increased as long as the Act was implemented. In the start, the main societal concerns were related to pesticide use, later other impacts become gradually included as for example benefits to the community/public utility, opportunities to reuse seed for farmers, effects on global agriculture structures, and North-South issues of equity.

In 2011 the Board initiated a project aimed at translating the concepts of sustainable development, social benefit and ethics in the Act. Two GM traits, insect-resistant genetically modified plants (2011) and herbicide-resistant plants (2014) were chosen as case studies. A group of interdisciplinary scientists and stakeholders was settled . The group elaborated several parameters (see Table 1).

Table 1. Parameters and questions to applicants included in the guidelines elaborated by the Norwegian Biotechnology Advisory Board for conducting SEC assessments (NBAB, 2011 and 2014).

Parameter	**Questions to Applicants**
Environment/Ecology	On the GM plant: characterization, gene flow, interaction between plant and the environment, preservation of biodiversity, comparison with control plants
	On the herbicide/Bt toxin: characterization, effects of altered use, development of resistance
	Soil, water, energy and climate
Society/Economy	The right to sufficient, safe and healthy food (food safety, security and quality)
	Animal health and welfare (feed quality)
	Living conditions and profitability for the farmers who cultivate GM plants, in the short term (less than 5 years) and in the long term (more than 20 years). Parameters include: health and safety, contracts and conditions, employment, developments of costs and incomes, agronomic factors, the right to seed, ownership rights *etc.*
	Plant genetic resources for food and agriculture
	Independent risk research
	Freedom to choose agricultural system in the future

One challenge for the broader assessment in Norway is that there is a lack of information and empirical data both in the scientific literature and in the application for approval of GMOs.

Options for inclusion of socio-economic considerations

Binimelis and Myhr (2016) found that countries that has included socio-economic considerations has chosen one of the following two options:

- Socio-economic considerations assessed in the general risk assessment procedure (considered to be part of the environment)
- Socio-economic considerations evaluated through an independent assessment (sometimes consecutive to the environmental and health risk assessment, others in a parallel but separated process).

The potential impacts that are to be addressed are both on the micro -level (e.g. farm, households) and at the macro-level (e.g. community or sector, country or region), and can include changes on lifestyle, work opportunities, economic revenue and human relationships etc. To address impacts is important to have measurement of changes as for example benefits and costs before the use and release of new technologies (ex ante) and after approval of the new technology (ex post). In addition, it is necessary to compare with baseline or alternative systems, for example for GM agriculture the most often comparison is done with monoculture and industrialized agriculture.

To have an appropriate comparator may be challenging with synthetic biology-based products and applications that opens up for completely new process ways for production of biological products or as well as the development of radical new products and applications. One option may therefore to instead of using a comparator assess the use of syntethic products and applications according to other parametres. This parametres may be found in national protection goals or in the national goals and can for example be assessment according to contribution to food production and food sovereignty, for the standards for levels of the use of pesticides or protection levels in nature and food by chemical residues, contribution to the f protection of biodiversity and wildlife, as well as human, animal and environmental health and welfare.

How to assess socio-economic impacts

There is at present no agreement on which framework or methods to choose when assessing socio-economic considerations. It is at present discussed what the appropriate scope and methods are, what the timing of considerations, the use of baselines, criteria, indicators, "endpoints" or targets should be, the role of public participation, relationships with other fields of knowledge, and the connection to other dimensions of risk and impact assessment such as environmental and health (Binimelis and Myhr 2016; Spök 2010).

Drawing on others work on broader criteria including socio-economic impacts (Rosendal and Myhr 2009, Catacora-Vargas 2014, NBAB, 2011 and 2014), a suggestion for questions to be explored for the assessment of socio-economic impacts by synthetic biology-based products and application could be:

- Does it solve a problem or a societal challenge?
- Does it impact the users and their needs?
- Does it impact the economic stability?
- Does it impact the environment?

Relevant research question for gathering empirical data to answer this can for example be:

- Is the new product/ production system facilitated by synthetic biology contributing to the improvement of health and nutrition?

    - For whom? How?
- Is the new product/ production system facilitated by synthetic biology strengthening job security and rural/ urban livelihoods?
    - For whom?
    - Is there important geographical considerations (national versus local levels)
- Is the new product/ production system facilitated by synthetic biology causing change in access to land, water seed, knowledge, market?
- Is the new product/ production system facilitated by synthetic biology impacting the economic stability?
    - Increased economic revenue
    - Reduced production costs
    - Increased market shares and/or trade
    - Implications by intellectual properties
- Is there impacts on the environment?
    - Increased or reduced pollution
    - Increased or reduced use resources
- Is there impacts on biodiversity and genetic diversity?
- Is there an alternative approach?

How to assess this questions require appropriate conceptual, methodological and empirical research followed with development and refinement of methods and frameworks for the analysis and presentation of the empirical data.

Recommendation

Socio-economic considerations need to assessed from case-to-case and may be dependent of purpose and context as well as national and regional conditions. Therefore, it is necessary to build competence in how to assess these impacts and considerations early on, even at the planning of research and development projects. This will also help to provide baselines and for identification of adequate comparators. The assessment itself assessments may result in contradictory and divergent results, and these can be between different socio-economic impacts and between socio-economic impacts and the environmental and health risk assessment. The reason for this is that the results will be dependent on to the framing used, the choice of assessment endpoints to be included, and the methods applied, and highlights the importance for transparency, openness and accuracy in the communication of the assessment process.

The assessment itself must be interdisciplinary and also involve stakeholder as regulators, members of civil society, NGOs and industry representatives. Hence there will also be a need to characterise the different roles played by stakeholders in the analysed regulatory frameworks at different phases of the assessment, e.g. at the beginning of the process so as to frame the issues, during the assessments so as to provide data, and at the end of the process for reviewing conclusions and providing opinions. Possible means for participation and/or consultation during socio-economic assessments need further elaboration.

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Ethical aspects and concerns raised by the use and application of synthetic biology-based products and applications

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Synthetic biology has been followed with discussions of ethical aspects and the need for broader assessment. This is acknowledged by both scientists within the field and by policymakers, and exemplified by the inclusion of ethical and social awareness through approaches as responsible research and innovation (RRI) driven by research funding agencies in Europe and by initiatives as the SynBERC in USA. The main ethical concern can be distinguished between those that are about how to handle scientific uncertainty of risk to health and the environment, and those that are not connected to the safety for human health and the environment.

Ethics

Ethics (moral philosophy) is a branch of philosophy that involves systematizing, defending, and recommending concepts of right and wrong conduct (http://www.iep.utm.edu/ethics/) . It is used to examine what is right and what is wrong, and sets norms and principles of right action. In general terms is can be said that there are two approaches to ethical decision making:

- Utilitarian approach – states that something is good if it is useful, and an action is moral if it produces the "greatest good for the greatest number"
- Deontological approach (Kantian approach or duty ethics) – focuses on certain imperatives, or absolute principles, which should be follow out of a sense of duty and which should dictate actions.

With emerging technologies ethics has got a more prominent role. It started with the sequencing of the human genome project in the USA, where it was discussed that the findings could have ethical implications. Studies on ethical, legal and social impacts (ELSI) was initiated, and this was also followed up in many other countries and expanded to include the use of bio- and gene technology in a variety of fields as for example in agriculture, for industrial processes etc. Relevant aspects for such studies has included human dignity and integrity, animal welfare, consumer acceptance, social benefit, solidarity, patent rights and benefit sharing, views on nature, biodiversity, sustainability, and how to understand risk and uncertainty. Recent years other issues have been included in ethical debates as for example researcher's responsibility and how to achieve robust technological development. The purpose by including ethics in such projects is to identify and to understand the perspectives of those impacted and those that may not be impacted, and to facilitate a dialogue for gaining enhanced understanding and/or informed compromises.

Ethical aspects and concerns with synthetic biology

There has been raised many ethical concerns related to the use of synthetic biology-based products and application. Here ethical issues by scientific uncertainty of harm, misuse of knowledge for purposes as bioterrorism, the possibility of overstepping human imitations, and the possibility for undermining the relationship between living things and machines will be briefly presented.

Synthetic biology: the same as genetic modification or more?

Although there is no unequivocal definition of synthetic biology it can be described as an engineering approach to design and construct biological compounds functions and organism not found in nature, or to change existing biological systems to perform new functions. Here are for example two definitions:

- *"Synthetic biology is a) the design and construction of new biological parts, devices and systems and b) the re-design of existing natural biological systems for useful purposes" (*http://syntheticbiology.org/FAQ.html)
- *Synthetic biology is "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms*" (EC, 2015)

During the Conference of the parties (COP 12) under the Convention of Biological Diversity in 2014 the parties was urged to take a precautionary approach to synthetic biology and to establish effective measures to regulate environmental release (CBD, 2014). It was also emphasised the importance of conducting risk assessments before approving field trials, and scientific assessments that consider risks to conservation and sustainable use of biodiversity as well as human health, food security and socio-economic considerations. One of the tasks that was highlighted for the Ad Hoc Technical Expert Group of synthetic biology was to:

- Identify the similarities and differences between living modified organisms (as defined in the Cartagena Protocol) and organisms, components and products of synthetic biology techniques to determine if they fall under the scope of the Cartagena Protocol,
- Identify if other instruments adequately regulate the organisms, components or products derived from synthetic biology techniques in so far as they impact on the objectives of the Convention and its Protocols;

Some of synthetic biology compounds and systems will fall under the definition of genetically modified organisms (GMOs), and thereby raise similar questions and issues of scientific uncertainty with regard to health and environmental safety. For example, in the Cartagena Protocol GMO (LMO) is: any LMO that possess a novel combination *of genetic material obtained through the use of modern biotechnology* (modern biotechnology covers also in vitro techniques including rDNA and cellfusion). In addition, synthetic biology will expand present approaches to allow for simultaneously change of a large number of nucleotides by gene synthesis, and to reconstruct as well as construct new organisms with synthetic genomes (König et al., 2013). Possibilities that present instruments used in risk assessment and risk management of GMOs will not be adequate for or appropriately cover, emphasising the importance to acknowledge the involved scientific uncertainty.

Scientific uncertainty: contained versus deliberate release

When considering scientific uncertainty and risk there is an important distinction between the use of synthetic biology at the contained level versus deliberate release.

- Contained use includes
  - Lab safety rules and implementation of physical, chemical and biological barriers to preclude the interactions with the environment
  - Biosecurity measures to avoid contamination

- Deliberate release is based on a:
  - Thorough case-by-case assessment of the potential environmental risks arising from release or escape
  - Biosafety measures to eliminate or minimize these risks

Contained use and deliberate release of synthetic biology products, as for example plants and microorganism, needs to be assessed case-by-case and in the context where the applications or products will be used. Within contained use, further development of containment strategies as xenobiotic mechanisms may be relevant (König et al., 2013). With regard to deliberate release, there will in safety assessment be a challenge to find a relevant comparator (EC, 2015), putting more stress on the importance of finding appropriate methods and models for risk assessment and for approaches to be used in monitoring and surveillance after approval for use.

Types of scientific uncertainties

Different academic disciplines, risk assessors and managers from various fields have different ways of understanding uncertainty and focus on different aspects of it. As a result, several typologies, taxonomies and ways of structuring different forms of uncertainties exist. Skinner et al. (2014) provide an useful overview of the many ways uncertainties are presented and understood, as well as the many tools and structures developed to analyse its various forms.

One of these typologies centres on the concepts of *risk, uncertainty, indeterminacy, ambiguity and ignorance* (see for example Stirling, 1998), and are briefly presented in Table 1. As illustrated in the table can types of uncertainties as risk and uncertainty that can be dealt with in conventional risk assessment, and that there are uncertainties as indeterminacy, ambiguity and ignorance that needs other approaches.

With synthetic biology-based products and applications it will therefore be necessary to both build on the work on risk assessment and risk management approaches and procedures undertaken at present with GMOs. This includes to elaborate on how present methods can be used adequately as they are or need to be expanded to acknowledge the novelties with synthetic biology, or if new methods and models need to be developed. In addition, it will be necessary to identify frameworks for uncertainty analysis that can be used.

Numerous methods and tools for characterisation and analysis of uncertainties have been developed (see for instance Skinner et al., 2014 for an overview). One example is the Walker & Harremöes (W&H) framework (Walker et al, 2003). The aim of this framework is to provide a conceptual framework for the systematic treatment of uncertainties in model-based decision support. Comprehensive analysis of uncertainties in environmental risk assessments of synthetic biology-based products and applications can be improved by the use of such uncertainty analyses, will help to facilitate communication of uncertainty to risk managers and decision-makers, and can also be used for making informed decisions about future options for research.

Type of uncertainties	Explanation	Approach/ Implications
Risk	We can imagine the range of possible hazards and calculate the probability of those hazards occurring, even though whether any of the hazards will occur or not remains unknown.	Can be dealt with through conventional risk assessment procedures.
Uncertainty	We can imagine the range of possible hazards, but we do not know the probabilities for their occurrence. It is however possible to calculate that probability, but we do not have enough knowledge to do so yet.	Can be dealt with through conventional risk assessments. More research should be initiated to reduce the level of inexactness.
Indeterminacy	For complex, open, interacting systems, it is impossible to include all the relevant factors and interactions in the calculations	Scientific findings must be treated as partial and conditional explanations, and therefore possibly fallible. Hence, we must expect and be prepared for surprises.
Ambiguity	We can variously frame both the impacts we are interested in and the way we approach, interpret and understand the knowledge and calculations generated about them.	To acknowledge the diversity of possible framings, negotiating across different ones where possible, and at least being transparent about the particular frames that are chosen and the reasons for their selection.
Ignorance	We cannot imagine the possible impact. Not only have we not yet calculated the probability of the event, we are unaware of what we should make calculations for. For instance, the inability to predict unintended effects.	To pursue a diverse range of policy options to maintain flexibility, resilience and reversibility, as well as to consistently and vigilantly monitor for potential surprises. General surveillance as a tool to address ignorance.

Table 1: Typology of uncertainties in policy-relevant science (adapted from Wickson et al. 2010)

Knowledge is misused (dual-use dilemma)

Synthetic biology has made gene technology more accessible for not trained scientists, with the cost of increased susceptibility for potential dual use. Dual use technologies have the potential to produce both desired and malicious products. One example is the use of microorganisms for production of medicines (as for example artemisinin against malaria) versus the use of pathogen microorganisms that are made more dangerous for bioterrorism purposes.

The potential to generate new pathogens by synthetic biology has raised concern both with regard to how screen for such purposes, how to train scientists within ethics to how one can constrain publication of scientific progress and understanding that can be used in bioterrorism purposes (Fauci and Collins, 2012). This has previously been discussed with GM vaccines, for example with the unexpected findings by Jackson and colleagues in 2001 when they were researching how to make efficient vaccines in biocontrol purposes based on mousepox (Evans, 2014).

In general, all scientific activity should strive for openness and transparency, also research that may have dual uses as for example bioterrorism since such research increase our understanding of unexpected findings and provides a basis for how to increase defence against the same pathogens.

More emphasis need to put on researcher responsibility with regard to knowledge that should be sought, and to build more awareness on ethical and other broader impacts by a by their projects.

Overstepping human limitations

With GMOs the purpose was to introduce genetic modification by removing, silencing or inserting genes. The terms used with synthetic biology includes designing and creating life. Also the term recreation has been used, and then in relation to bring extinct animals back to life as for example the mammoth.

This potential has raised ethical concerns related to naturalness, ethical questions related to the relationship between humans and other living organisms and the moral status of the products made by synthetic biology (Nuffield Council of Bioethics, 2015; Schmidt et al., 2009). The Nuffield Council of Bioethics has in their project in naturalness identified that the term was used in many different connections with new technologies and that a variety of words was used to convey ideas about naturalness, such as normal, pure, real, organic, unprocessed, and artificial, and synthetic. They also found that the terms natural, unnatural and nature are often used as common term for a range of different values that are meaningful or worth protecting to people.

Playing God can also be considered as a placeholder or a methaphore used in the debate concerning synthetic biology and can both have secular and a religious interpretation:

- Secular interpretation
  - Overstepping human limitations in evolutionary processes
  - Fail to recognise human limitations by overestimating ability to control complexity
  - Tampering with nature can have unexpected consequences.
- Religious interpretation
  - Not dependent on a natural template
  - Create life from non-living material
  - Goes against the will of God or distort God´s creation

Especially the NGO ETC has used the term Playing God and Pat Mooney from ETC has claimed that "for the first time God has competition". The Church of Scotland (2010) has stated that this religious interpretation and argument against the use of synthetic biology is not valid since God creates *ex nihilio*, out of nothing. The conception of what is natural and what is in competition with God may change over time. However, both naturalness and the "Playing God" terms are expressions used as placeholders for that the use of synthetic biology may involve risks to nature and that the scientist do not have control or do not know what they are doing. Moreover, that synthetic biology may affecting the moral status of living things and the dignity of life.

It is therefore important that both scientists, research funding agencies and policy makers understand and acknowledged these values and beliefs if they wont to pursue openness and wants to take into account the views of the public in research and when developing policies for science, technology and medicine. Again this links up to the responsibility of scientists for providing information and for the use of new technology for the benefits of humans and the environment.

Undermining the distinction between living things and machines

Development and creating new life entities from biological components as for example minimal genomes may lead to a mechanistic understanding of life and reduced awareness of biological interactions that constitutes the complexity of ecosystems (Ainsley and Newson, 2011). Terms used to describe synthetic biology includes biobricks, minimal cell, living machines etc. Indicating that function of the synthetic biology-based products are decided and controlled by an external agent – the scientist. Hence a mechanistic description of life without a link to biological interactions and complexity (Ansley and Newson, 2011), and a concern that this will further expand the patenting of biology. This is also in contrast with the description of living things, that their function includes self-maintenance and organisation. Function and moral status are complicated issues and includes both philosophical and cultural aspects (Deplazes and Huppenbauer, 2009; Schark, 2012), that needs further elaboration.

Summary

It is crucial that ethical aspects are taken into account and that scientists, policymakers takes initiatives for information sharing as well as initiatives for dialogue. Such initiatives needs to explore the:

- Problem solving nature of the new product or application or by synthetic biology itself
- Environmental issues (biodiversity, pollution, resources)
- The knowledge basis (scientific uncertainty, risk and precaution)
- Users and their needs (welfare and wellbeing)
- Impacts on non-users
- Metaphores and placeholders as “Nature”, “naturalness”, “machines” and Playing God
- Intellectual property rights
- Institutional structure and representation in decision-making processes

Also further elaboration on what the term safe constitutes with synthetic biology and what responsible research and use are together with elaboration of who the stakeholders are and should be involved in making decisions about approval of products and applications. SHuch discussions and dialogues will not create consensus, but will make a broader platform for making decisions.

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Relevance and Application of the Cartagena Protocol on Biosafety to Synthetic Biology

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Cartagena Protocol on Biosafety

Efforts to establish legally-binding rules on genetically modified organisms (GMOs) were first introduced onto the international agenda during the discussions leading to the Rio Earth Summit. Finalized in 1992, the Convention on Biological Diversity (CBD), in its Article 19(3), provided governments the mandate to consider the need for a protocol on biosafety to address the risks of genetic engineering.

After long and at times acrimonious negotiations, the Cartagena Protocol on Biosafety was finally concluded in 2000. It entered into force on 11 September 2003 after obtaining the requisite number of ratifications, acceptances, approvals, or accessions. It is the first and only international law to specifically regulate genetic engineering and GMOs. (In the Protocol, GMOs are known as living modified organisms or LMOs.)

The Cartagena Protocol is legally binding in the international legal system and in the legal systems of countries that have ratified, approved, accepted, or acceded to it. As of October 2016, there were 170 Parties to the Protocol.

The Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety is a separate treaty that deals with the issue of liability and redress for damage resulting from the transboundary movements of LMOs.

Significance of the Cartagena Protocol

For the first time in international law, there is recognition that LMOs are inherently different from other naturally occurring organisms and may carry special risks and hazards, and therefore need to be regulated internationally. The Protocol addresses the fact that LMOs may have biodiversity and human health impacts, and that these impacts need to be risk assessed. The Protocol also recognizes that socio-economic considerations can be taken into account when making decisions on LMOs, an issue that is particularly significant for developing countries.

Importantly, the Cartagena Protocol puts the Precautionary Principle into operation in decision-making (i.e. in the absence of scientific certainty, a party should err on the side of caution and could restrict or ban the import of LMOs on account of their potential adverse effects) and this further establishes the Principle in international law.

The Protocol deals mainly with the transboundary movement (import and export) of LMOs, including illegal and unintentional transboundary movements. However, its scope extends to all kinds of LMOs, including plants, food, pharmaceuticals, animals, insects, trees, for industrial use, etc.

Its 'advance informed agreement' (AIA) procedure, governs the first transboundary movement between Parties, of LMOs for intentional introduction into the environment. This procedure essentially establishes the principle of prior informed consent, that there should be no export of LMOs unless the importing country approves its transboundary movement. It also establishes the right of the importing Party to say 'no' to a given request for import.

The AIA procedure involves three key steps. First, the Party of import must be notified by the Party of export or the exporter, of the latter's intent to send LMOs. Thus, countries now have an international right to be notified that a LMO is going to be shipped to them.

The Party of import then evaluates the risk assessment which has been submitted by the Party of export or exporter, or alternatively conducts its own risk assessment if it is not satisfied with the risk assessment submitted, which is usually conducted by the developer of the LMO. Risk assessment can take into account the expert advice of, and guidelines developed by relevant international organizations. Precaution is also one of the general principles of risk assessment.

Finally, the Party of import makes its decision based on precaution. The decision could be for unconditional approval, approval with conditions, prohibition, a request for additional relevant information or extension of the time period for further consideration of the application.

The AIA procedure thus places obligations on exporting Parties, to first seek the informed approval of importing Parties before any transboundary movement can occur. It reverses the burden for importing countries that have little capacity and information to know what is entering into their territories, and to regulate them accordingly. It also affords rights and places corresponding obligations on to importer countries.

However, the Protocol excludes some LMOs – LMOs is transit, in contained use, and that are intended for food, animal feed or for processing – from the AIA procedure. Nonetheless, they are still covered by the Protocol and all other provisions apply to these categories of LMOs. For LMOs that are intended for food, animal feed or for processing a separate procedure applies; countries that make a final decision on domestic use must notify the Biosafety Clearing House (BCH).

Parties to the Protocol can moreover choose to implement the AIA procedure at the national level in relation to *all* LMOs. Within the domestic regulatory system, this principle can also apply to nationally developed LMOs that undergo an approvals process.

Definitions[1]

In order to determine whether or not the organisms, components and products of synthetic biology are addressed by the Cartagena Protocol on Biosafety, it is instructive to explore further some of the definitions under both the Protocol and its parent treaty, the CBD. (See Figure 1 for a schema showing these definitions and their relationship to each other).

[1] In this section, the interpretations and implications of the text of the Cartagena Protocol are taken from *An Explanatory Guide to the Cartagena Protocol on Biosafety.*

In Article 2 of the CBD, *"Biotechnology"* means "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". Many of the examples of organisms developed though synthetic biology can thus be considered as "living modified organisms resulting from biotechnology" as defined by the CBD.

Article 3 of the Cartagena Protocol meanwhile provides three definitions that are interrelated and have to be read together: *"living modified organism", "living organism", and "modern biotechnology".*

Since the scope of the Protocol (Article 4) applies to "all living modified organisms", we need to understand how these are defined in the Protocol.

"Living modified organism" means "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology".

A living modified organism is thus defined in the Protocol to include only those living organisms that

- contain novel combinations of genetic material; and
- have been produced using the techniques of modern biotechnology.

"Living organism" means "any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids". The specific mention of viruses, viroids and sterile organisms ensures that such entities – which cannot actively replicate genetic material or reproduce through sexual reproduction – are also covered by the Protocol. Plasmids and naked DNA are not included, but where a novel combination of genetic material is introduced through the use of naked DNA or plasmids through modern biotechnology, then the resultant organism would qualify as an LMO. Similarly, the definition would cover a living organism in which a plasmid created by modern biotechnology and that contains a novel combination of genetic material is present, even where the plasmid is not integrated into the chromosomes of that organism.

While the Cartagena Protocol does not define *"genetic material"*, the CBD does: "any material of plant, animal, microbial or other origin containing functional units of heredity". Functional units of heredity are understood to be nucleic acids containing genetic information. These nucleic acids may be of plant, animal, microbial or other origin. In addition, the definition also covers *an*y material of plant, animal, microbial or other origin, such as whole organisms or parts of organisms, which contains nucleic acids that contain genetic information. In the context of the Cartagena Protocol, genetic material can be understood to refer to nucleic acids that contain functional units of heredity.

A "novel combination of genetic material" can be regarded as a combination that was not previously known to exist at the time it was first produced. Linked to the CBD definition of genetic material, this can then be understood to refer to a novel combination of nucleic acid containing functional units of heredity. It is important to note that the novel combination relates solely to a combination of genetic material, even if this does not result in an observational change.

The novelty of a combination could arise through a novel form of a functional unit of heredity, e.g. resulting from a change that modifies the overall sequence of nucleotides within the unit, whether

by altering, inserting or deleting one of more nucleotides. Novelty could also arise from a novel arrangement of functional units of heredity e.g. introduction of genetic material from different species, or rearrangement of genetic material of the same species. A novel combination could arise from a single change in a nucleotide sequence or from much larger changes.

According to the Cartagena Protocol, the novel combination of genetic material must be "obtained through the use of modern biotechnology". This is a fundamental criterion for the definition of a LMO. Whether or not an organism is an LMO under the Protocol depends on whether "modern biotechnology" is used to create a novel combination of genetic material.
Furthermore, even if the novel combination of genetic material obtained through modern biotechnology is subsequently transferred into another organism through traditional breeding or selection techniques, the resulting organism is also an LMO under the Protocol. A good example of such LMOs are stacked LMOs as a result of crosses between two or more LMOs.

"Modern biotechnology" is defined in the Cartagena Protocol as:

"The application of:

a) In vitro nucleic acid techniques, including recombinant 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".

This therefore, includes, but is not limited to, in vitro nucleic acid techniques applied to the insertion, deletion and alteration of genetic material. The two qualifications are that natural physiological reproductive or recombination barriers must be overcome, and that they are not techniques used in traditional breeding and selection.

The negotiators of the Cartagena Protocol recognized that any definition of modern biotechnology should cover new techniques not yet envisaged at the time that the Protocol was adopted, but which may emerge in the future. This is because the technology is developing all the time, and the legal instrument had to be drafted so as to not exclude new technological processes not yet identified but which may give rise to novel combinations of genetic material through the use of modern biotechnology. Therefore the definition in Article 3(i) seeks to reflect the need to cover future techniques, by using the wording "in vitro nucleic acid techniques", giving two *existing* examples i.e. recombinant DNA and direct injection of nucleic acids, and leaving open whether new techniques will be regarded as "in vitro nucleic acid techniques" or not, and by referring to fusion of cells.

How does the Cartagena Protocol on Biosafety apply to synthetic biology?
Given the discussion above, and the definitions contained both in the CBD and the Cartagena Protocol, it is clear that these definitions would apply to most of the living organisms resulting from current synthetic biology techniques. This means that the relevant provisions of both the CBD and the Cartagena Protocol would apply to synthetic biology.

Under the CBD, its biosafety provisions relating to LMOs are found in Article 8(g), 19(3) and 19(4). Under Article 8(g), where LMOs resulting from biotechnology are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account risks to human health, Parties are required, as far as possible and as appropriate, to establish or maintain means to regulate, manage or control these risks at a national level. Article 19(3) was the enabling provision that gave rise to the Cartagena Protocol by obliging Parties to consider the need for and modalities of a protocol in the field of the safe transfer, handling and use of LMOs. Article 19(4) obliges Parties to provide any available information about the use and safety regulations in handling LMOs, as well as any available information on the potential adverse impact of the specific organisms concerned to a Party into which these LMOs are to be introduced.

At the same time, all the provisions of the Cartagena Protocol apply to living organisms resulting from synthetic biology that fulfil the criteria of possessing a novel combination of genetic material and obtained through the use of modern biotechnology.

Therefore, discussions on synthetic biology have been on-going under both the CBD and the Cartagena Protocol. In particular, the CBD established an Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology in 2014. The AHTEG, which met in September 2015, agreed an operational definition of synthetic biology, to assist Parties in their implementation of the provisions of the CBD: "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".

The AHTEG also agreed that living organisms developed through current and near future applications of synthetic biology are similar to LMOs as defined in the Cartagena Protocol.

One issue to note is that of components and products of synthetic biology, given that the scope of the Cartagena Protocol applies to *living* modified organisms. The CBD Secretariat and AHTEG on Synthetic Biology refer to "components" as parts used in a synthetic biology process (e.g. a DNA molecule), and "products" as the resulting output of a synthetic biology process (e.g. a chemical substance), and considers "components" and "products" as non-living.

However, the Cartagena Protocol does address "products thereof" in a limited way, under provisions and annexes addressing information sharing and risk assessment. Products thereof are "processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology". For example, specific compounds produced by microorganisms that have been altered by synthetic biology techniques may also fall within the Protocol's definition of "products thereof" if they contain nucleic acids containing a novel combination of genetic material.

Article 20 requires Parties to make the summaries of risks assessments, including relevant information regarding products thereof, available on the Biosafety Clearing-House, the information-sharing website administered by the CBD Secretariat. Annex I, which details the information required

in notifications, includes products thereof, while Annex III which is the Protocol's general framework on risk assessment, is applicable to products thereof.

Likewise, while naked DNA and its constituent parts resulting from synthetic biology are not included in the definition of living organisms (see earlier discussion) under the Cartagena Protocol, they would be addressed as "products thereof" if they contain detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology. Furthermore, if novel DNA is inserted into living cells for shipment, the cells themselves would qualify as "living organisms" and hence be covered by the Protocol, as they would contain novel combinations of genetic material and would have been produced using the techniques of modern biotechnology.

In any case, national laws may specifically regulate products and components of synthetic biology. It is worth recalling that the Protocol is a negotiated international law framework that sets minimum standards for national biosafety implementation. This is clearly established in Article 2(4) of the Cartagena Protocol: "Nothing in this Protocol shall be interpreted as restricting the right of a Party to take action that is more protective of the conservation and sustainable use of biological diversity than called for in this Protocol, provided that such action is consistent with the objective and the provisions of this Protocol and is in accordance with that Party's other obligations under international law." Sovereign countries interpret and implement the Cartagena Protocol, and can do so in a comprehensive manner, and with higher standards for biosafety.

At the current stage of development of synthetic biology, many of the applications are still at the laboratory research stage. It is thus also worth remembering that Article 6 of the Protocol, while exempting LMOs destined for contained use from the AIA procedure, preserves the right of Parties to subject all LMOs to risk assessment prior to decisions on import and to set standards for contained use within its jurisdiction.

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Synthetic Biology and Relevant International Laws: Gaps and Overlaps

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Introduction

'Synthetic biology' as such has not been addressed specifically in the text of any multilateral treaties. However, there are a multitude of treaties, customary rules and general principles of law, as well as other regulatory instruments and mechanisms, which could apply to all or some forms of synthetic biology.

The treaties could apply to issues such as:

- The transfer and handling of components, organisms and products resulting from synthetic biology techniques;
- The use of components, organisms and products resulting from synthetic biology techniques for a specific purpose, in particular for hostile purposes or in armed conflict;
- Intellectual property rights associated with components, organisms and products resulting from synthetic biology techniques e.g. patentability; and
- Access to genetic resources used in synthetic biology techniques, and sharing of benefits arising from their utilization.

The Secretariat of the CBD produced a comprehensive publication addressing the potential impacts of synthetic biology on biological diversity, and the gaps and overlaps with the provisions of the Convention and other agreements. This document was published in 2015 as CBD Technical Series No. 82. This chapter summarises the key findings of the document on the international regulatory regime applying to synthetic biology. It aims to provide an overview of the international treaties and fora that are relevant, and where the gaps are still remaining.

Synthetic biology, the CBD and the Cartagena Protocol on Biosafety

From the discussion on definitions in the preceding Chapter, the CBD and its Protocols have a clear and overarching mandate on synthetic biology.

Convention on Biological Diversity

In terms of the conservation and sustainable use of biological diversity, Article 14 of the CBD obliges Parties to conduct environmental impact assessment for activities that are likely to have significant impacts on biological diversity with a view to avoiding or minimizing such effects.

The biosafety provisions regarding "living modified organisms resulting from biotechnology" are in Articles 8(g), 19(3) and 19(4) of the CBD, as discussed in the preceding Chapter, and would therefore apply to synthetic biology. These broadly oblige Parties to establish or maintain means to regulate,

manage or control risks at a national level, ensure safe transfer, handling and use, and provide available information about the use and safety regulations and potential adverse impacts.

As such, synthetic biology has been discussed under the Convention on Biological Diversity (CBD) since 2010. In Decision X/13, Parties, other Governments and relevant organizations were invited to apply the precautionary approach to the field release of synthetic life, cell, or genome into the environment.

In 2012, Decision XI/11 recognized 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. The decision urged Parties and invited other Governments to take a precautionary approach when addressing threats of significant reduction or loss of biological diversity posed by synthetic biology organisms, components and products. It also noted, based on the precautionary approach, the need to consider the potential positive and negative impacts of synthetic biology components, organisms and products, and initiated a process by which synthetic biology could be considered by the CBD's Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA).

A precautionary approach to synthetic biology was again reaffirmed in 2014. Decision XII/24 further urged Parties and invited other Governments, inter alia, to establish, or have in place, effective risk assessment and management procedures and/or regulatory systems to regulate environmental release of any organisms, components or products resulting from synthetic biology; to approve organisms resulting from synthetic biology techniques for field trials only after appropriate risk assessments have been carried out; and to carry out scientific assessments of synthetic biology organisms, components and products that consider risks to conservation and sustainable use of biodiversity as well as human health, food security and socio-economic considerations; and that such assessments should be done with, where appropriate, the full participation of indigenous and local communities.

The issue of synthetic biology is once again on the agenda for the CBD Conference of the Parties (COP13) in December 2016. Work has progressed with the establishment of the Ad Hoc Technical Experts Group (AHTEG) on Synthetic Biology (see Box) and the issue will continue to be discussed and elaborated in the coming years, particularly as COP13 is asked to extend the mandate of the current AHTEG.

Ad Hoc Technical Experts Group (AHTEG) on Synthetic Biology

The CBD Conference of the Parties (COP) in 2014 established an Ad Hoc Technical Experts Group (AHTEG) on Synthetic Biology. Preceded by an online forum that involved hundreds of experts to discuss key issues, the AHTEG met in September 2015. A peer-review of the AHTEG outcomes was held in November 2015 and the AHTEG recommendations were considered by the twentieth meeting of SBSTTA in April 2016.

The Terms of Reference for the AHTEG asked it to, among others:
Identify the similarities and differences between LMOs (as defined in the Cartagena Protocol) and organisms, components and products of synthetic biology techniques to determine if LMOs derived from synthetic biology fall under the scope of the Cartagena Protocol;

Identify if other national, regional and/or international instruments adequately regulate the organisms, components or products derived from synthetic biology techniques in so far as they impact on the objectives of the Convention and its Protocols;

Work towards an operational definition of synthetic biology;

Identify the potential benefits and risks of organisms, components and products arising from synthetic biology techniques to the conservation and sustainable use of biodiversity and related human health and socioeconomic impacts relevant to the mandate of the Convention and its Protocols;

Building on the work on risk assessment and risk management undertaken by the Cartagena Protocol, compile information on best practices on risk assessment and monitoring regimes currently used; and

Identify if the existing arrangements constitute a comprehensive framework in order to address impacts of organisms, components and products resulting from synthetic biology relevant to the objectives of the CBD and its Protocols, in particular threats of significant reduction or loss of biological diversity.

Cartagena Protocol on Biosafety

CBD Technical Series No. 82 stresses that living organisms resulting from current synthetic biology techniques fall under the definition of "living modified organisms" under the Cartagena Protocol for Biosafety. Currently, as living organisms resulting from synthetic biology techniques fulfill the criteria of (i) being a living organism, (ii) possessing a novel combination of genetic material, and (iii) resulting from the use of modern biotechnology, the Cartagena Protocol on Biosafety is fully applicable to them. Therefore, its requirements pertaining 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, apply. (See the preceding Chapter for a more detailed discussion on the Cartagena Protocol).

This may need to be reassessed if and when future technological advances of synthetic biology lead to the creation of living organisms possessing novel combinations of genetic material, which are heritable and do not result from the use of *in vitro* nucleic acid techniques or cell fusion.

It should also be noted that the AHTEG on Synthetic Biology, established by the CBD Parties, has agreed that living organisms developed through current and near future applications of synthetic biology are similar to LMOs as defined in the Cartagena Protocol.

While the conversation on the components and products of synthetic biology under the Cartagena Protocol is more nuanced (see preceding Chapter for a more detailed discussion), it should be noted that they do in any case fall within the scope of the CBD and its objectives.

The Cartagena Protocol does contain some limited exemptions of some LMOs from some provisions. The Protocol does not apply to the transboundary movement of LMOs which are pharmaceuticals for humans that are addressed by other relevant international agreements or organizations (Article 5). Some examples of LMOs produced through synthetic biology which are pharmaceuticals for humans are live virus vaccines. However, as none of the organisms currently produced through synthetic biology which are intended to be used as pharmaceuticals for humans are directly addressed by

other relevant international agreements or organisations, they therefore would arguably fall under the Cartagena Protocol's scope.

Moreover, where synthetic biology organisms are used as 'biofactories' to produce pharmaceuticals such as in the case of artemisinin, the organisms themselves are not pharmaceuticals, but they are still LMOs produced by synthetic biology and would therefore be covered by the Cartagena Protocol. LMOs produced by synthetic biology that are pharmacueticals for animals would clearly not be exempted from the Protocol.

Some organisms resulting from synthetic biology techniques may fall under exemptions from the Cartagena Protocol's Advanced Informed Agreement provisions for LMOs, for example, if they are in transit, intended for contained use or for direct use as food or feed, or for processing.

Nonetheless, Article 6 of the Protocol preserves the right of a Party to regulate the transport of LMOs through its territory, and to subject all LMOs to risk assessment prior to decisions on import and to set standards for contained use within its jurisdiction. Similarly, a Party may take a decision on the import of LMOs intended for direct use as food or feed, or for processing, under its domestic regulatory framework that is consistent with the objective of the Protocol. Many such national frameworks require Advance Informed Agreement for LMOs intended for direct use as food or feed, or for processing.

In addition, once entered into force, the Nagoya–Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety will require Parties to provide at the national level for rules and procedures that address damage from LMOs, including those resulting from synthetic biology techniques, where such damage falls under the definition set out in Article 2 of the Supplementary Protocol. It is possible that LMOs resulting from synthetic biology techniques could cause adverse effects on the conservation and sustainable use of biological diversity, as described in CBD Technical Series No. 82.

Other international treaties relevant to synthetic biology

Treaties that address specific uses

Biological Weapons Convention

The Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxins Weapons and on their Destruction addresses microbial or other biological agents or toxins, including those that are components, organisms and products resulting from synthetic biology techniques. It provides a forum where further guidance for this aspect of synthetic biology could be developed.

The core obligation is for Parties to never in any circumstances develop, produce, stockpile or otherwise acquire or retain microbial or other biological agents or toxins that have no justification for prophylactic, protective or other peaceful purposes. Given that synthetic biology has the potential for dual use, the issue has been discussed explicitly under the Biological Weapons Convention. However, as of 2015, no concrete steps toward the development of an oversight

framework, guiding principles or models to inform risk assessment and oversight of scientific research have been undertaken.

SPS Agreement
Some applications of synthetic biology could also, 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; or as risks to human or animal life or health arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs.

If this is the case, measures taken by members of the World Trade Organization (WTO) to address these risks would count as sanitary and phytosanitary measures in the sense of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and would have to comply with the requirements thereof. Any measures taken would have to be based on a risk assessment and scientific principles, must not unjustifiably discriminate on other WTO members' exports and must not be more trade-restrictive than necessary to achieve the appropriate level of protection.

The SPS Agreement explicitly recognizes the international standards, guidelines and recommendations developed by the Codex Alimentarius Commission, World Orgnanisation for Animal Health (OIE) and the International Plant Protection Convention. The standards set by these bodies may be relevant to components, organisms and products resulting from synthetic biology.

Guidance exists as to the application of the standards to LMOs, although it is not clear how these standards could be applied for all forms of synthetic biology techniques. The standard setting organizations have not, as yet, explicitly addressed synthetic biology.

Treaties that address access and benefit-sharing

Convention on Biological Diversity
In the cases where synthetic biology requires access to genetic resources, the access and benefit-sharing requirements of the CBD would, in general, apply and thus require prior informed consent (unless otherwise determined) and the negotiation of mutually agreed terms. Parties are also obliged to take legislative, administrative or policy measures, with the aim of sharing in an equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Party providing such resources.

Nagoya Protocol on Access and Benefit-Sharing
Synthetic biology applications may also be considered as a way of utilizing genetic resources, as defined in the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization. National implementation and the negotiation of mutually agreed terms would assist parties to access and benefit-sharing agreements to clarify to what extent of the value chain the obligations to share benefits would continue to apply to the organisms, components and products of synthetic biology, including derivatives and their subsequent applications.

Components used in synthetic biology include virtual/digital information on functional units of heredity. In this context, CBD Technical Series No. 82 states that it is not clear whether virtual/digital information about genes and other genetic elements can be considered "genetic resources" or "genetic material" in accordance with the definitions contained in Article 2 of the Convention. It is also unclear whether the components used in synthetic biology and the products thereof may be considered "genetic resources" as defined by the Convention.

However, the combination of faster genome sequencing with rapid DNA synthesis and powerful gene editing techniques is creating new avenues for biopiracy that must be urgently addressed. The combination of these synthetic biology techniques could undermine implementation of the CBD's access and benefit sharing obligations, including the Nagoya Protocol. Genetic resources – whether DNA sequence of specific interest or even entire microorganisms and other small genomes – may now be transferred digitally and synthesized into living matter without physical exchange of biological material. This issue needs to be urgently addressed and will be discussed at COP13 in December 2016.

International Treaty on Plant Genetic Resources for Food and Agriculture
With regard to access to plant genetic resources for use in synthetic biology processes and the sharing of the benefits arising from commercialization, the International Treaty on Plant Genetic Resources for Food and Agriculture may be particularly relevant. The Treaty is recognized as one of the complementary instruments that constitute the international regime on access and benefit-sharing. The Treaty's Multilateral System for Access and Benefit-Sharing covers plant genetic resources for food and agriculture listed in its Annex 1. According to CBD Technical Series No. 82, some of these Annex 1 crops are the focus of synthetic biology research.

Treaties that address intellectual property

TRIPS Agreement
In accordance with the WTO's Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement), patents should be available under national law of WTO members (other than least developed countries) for innovative products and techniques in the field of synthetic biology, provided that they constitute inventions that comply with the general patentability standards.

Select products of synthetic biology techniques may fall under the subject matter exclusions provided by Article 27, paragraphs 2 and 3 of the TRIPS Agreement and may therefore be excluded from patentability by some WTO members. Paragraph 2 of Article 27 allows WTO members to provide this exclusion if it 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. Some synthetic biology applications may well meet these criteria in some countries and could provide grounds for exclusion from patentability.

Furthermore, paragraph 3 of Article 27 of the TRIPS Agreement allows WTO Members to exclude the following from patentability: diagnostic, therapeutic and surgical methods for the treatment of plants and animals; plants and animals other than microorganisms, and essentially biological

processes for the production of plants or animals other than non-biological and microbiological processes.

UPOV Convention

The results of current synthetic biology research that is focused on modifying existing "natural" genomes could also qualify for the "breeder's right" (a form of protection for intellectual property on plant varieties) under the International Convention for the Protection of New Varieties of Plants (UPOV).

Gaps in the current regulatory framework

The preceding section, drawing on CBD Technical Series No. 82, provided a brief overview of the numerous international treaties that are applicable to various aspects of synthetic biology.

From the discussion above, it is clear that the components, organisms and products resulting from synthetic biology would fall under the scope of a number of international 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 to ensure the conservation and sustainable use of biodiversity, and the fair and equitable sharing of the benefits arising from the utilization of genetic resources.

The CBD and its Protocols provide fairly comprehensive coverage but there are still gaps remaining. Work needs to continue in these fora to fully articulate to what extent they apply to synthetic biology, and how implementation should proceed. Such discussions are on-going in the AHTEG on Synthetic Biology under the CBD, and the AHTEG on Risk Assessment and Risk Management under the Cartagena Protocol, and synthetic biology will clearly continue to be discussed at the meetings of the CBD and its Protocols.

CBD Technical Series No. 82 further recommends that discussions in international fora may be needed with a view to identifying the gaps identified in an appropriate, consistent, comprehensive and adaptive manner. This could include a need to consider how to address potential impacts of very low probability, but with very high magnitude. Further discussions may also be needed if and when the advances in synthetic biology lead to the emergence of new gaps.

While some general principles of international law such as the duty to avoid transboundary harm, and the need to conduct an environmental impact assessment (EIA), 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, this would still form an incomplete basis to address all potential negative impacts.

A number of treaties exist which, in general, provide for mechanisms, procedures or institutions that can address potential negative effects associated with the application of synthetic biology techniques. However, there is no specific guidance for their application.

Even though the requirements of the Cartagena Protocol apply to most, if not all, organisms resulting from current synthetic biology techniques, it may still be necessary, for example, to identify elements of risk assessment methodologies that would be specific for living organisms developed through synthetic biology in order to ensure the effective application of its risk assessment provisions.

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Principles for a Holistic Regulatory Approach to Synthetic Biology

Lim Li Ching, Third World Network

Potential adverse effects of synthetic biology

The issue of regulation arises out of the need to avoid or minimize the potential adverse effects that could occur from the release of organisms, components or products of synthetic biology. These effects could be direct or indirect, intended or unintended, as well as immediate or delayed effects. The effects could occur at the genetic, population, or ecosystem level.

The Ad Hoc Technical Expert Group on Synthetic Biology in 2015 grouped the following (non-exhaustive) effects according to the impacts on the three objectives of the CBD:

Objective 1: Conservation of biological diversity

- Engineered fitness advantage may lead to invasiveness
- Enhanced gene flow that leads to loss of biodiversity
- Increased pathogenic potential
- Increased levels of toxic substances, which may be disruptive to soil, food-webs, and pollinators
- Negative effects on non-target organisms, such as pollinators
- Changes in organisms on the level of basic metabolic pathways, such as altered photosynthesis pathways, carbohydrate metabolism or nitrogen fixation, which may lead to changes in agricultural practice and land-use
- Applications aimed at altering and replacing natural populations (for example, gene drive systems) may have adverse effects at the ecosystem level

Objective 2: Sustainable use of biological diversity

- Increased demand for biomass crops, as well as changes in patterns of extraction of biomass, minerals and other sources of energy, may lead to changes in land use
- Replacement of natural products may lead to changes in the agricultural practices of communities, which may adversely affect traditional crops, practices and livelihoods
- Gene flow may lead to adverse effects on agrobiodiversity

Objective 3: Equitable sharing of the benefits of biological diversity

- Loss of market share and income by indigenous/local communities due to altered exploitation of genetic resources
- Shift in the understanding of what constitutes a genetic resource and the implications thereof, such as the misappropriation of the original source of the DNA information and, if benefits are derived from the use of such DNA information without prior informed consent and mutually agreed terms, the fair and equitable sharing of the benefits would not be possible
- Inappropriate access without benefit-sharing due to the use of sequenced data without material transfer agreements under the Nagoya Protocol

- Patent-driven and open-source approaches to synthetic biology may have different implications in the context of access and benefit sharing
- Indigenous peoples and local communities will not necessarily support or benefit from the utilization of genetic resources in synthetic biology

Challenges for risk assessment

Given the potential adverse effects of synthetic biology, risk assessment becomes a central issue. As the current and near-term (5-10 years) applications of synthetic biology build on techniques of modern biotechnology to create organisms with novel combinations of genetic material, it is expected that the general risk assessment methodology for LMOs would be applicable to organisms developed through synthetic biology.

However, while the potential adverse effects of synthetic biology are similar to those of 'classical' genetic engineering, it can be expected that the former will be broader and more intense due to the ability of synthetic biology to engineer more complex systems for use in a wider range of applications. Moreover there are likely to be higher levels of scientific uncertainty associated with synthetic biology, due to the higher level of complexity involved. For example, synthetically modified organisms are likely to have larger segments of modified DNA or even complete novel genomes. Synthetic biology could also lead to the development of new biological systems that do not exist in nature.

In future, organisms could be developed through synthetic biology that will fundamentally differ from naturally occurring organisms, making it impossible to conduct risk assessments based on a comparative principle, due to the lack of appropriate comparators.

Risk assessment may therefore be more challenging for synthetic biology, as the complexity of organisms increases, as novel gene sequences are more significantly modified, and as genetic components are assembled from a greater variety of sources. Future developments in synthetic biology will further raise specific challenges and limitations with regard to risk assessment principles and methodologies that are currently applied to evaluate LMOs. For example, if and when future commercial synthetic biology applications evolve to use techniques that do not rely on *in vitro* manipulation of nucleic acids to cause inheritable changes, current LMO risk assessment methodologies may no longer be suitable.

In practice, while existing approaches of risk assessment, management and communication can be used as a basis for assessing and mitigating the impacts of synthetic biology organisms, guidelines and methodologies would need to be developed and made available to address the additional uncertainties and knowledge gaps. As such, risk assessment methodologies that are currently in use will need to be revised and adapted to ensure that the risks of synthetic biology are adequately addressed. Specific consideration will also likely be needed to identify any gaps that exist in the current LMO risk assessment methodologies, and guidance needed on how to fill such gaps.

Due to the complexity and novelty of the organisms developed through synthetic biology, the type and depth of information that may be required to assess their risks will differ from the information typically provided by developers for conducting risk assessments of LMOs. The availability of appropriate (case-specific) scientific data is crucial for an adequate risk assessment of organisms, components and products of synthetic biology with a potential for adverse effects or an unknown level of risk for unintended effects. The general challenge is to keep up with the rapid pace of development. Efforts should be made to address the relevant risk issues by appropriate biosafety research. There may also be a need for a revised risk assessment framework to address the possible novel risks posed by products of synthetic biology whereby no parent organism can be used as comparators.

Given the acknowledged challenges for risk assessment that could be posed by synthetic biology, the AHTEG on Risk Assessment of Living Modified Organisms, established under the Cartagena Protocol on Biosafety, discussed the issue in 2016. This was preceded by discussions in the Open-Ended Online Expert-Forum on Risk Assessment and Risk Management.

The AHTEG developed an outline of guidance on "Risk Assessment of LMOs developed through synthetic biology", and the issue will be discussed at the Eighth Meeting of the Parties serving as the Conference of the Parties (COP-MOP8) to the Cartagena Protocol in December 2016. Parties to the Protocol are asked to consider establishing a process for the development of guidance on the basis of the outline developed, in coordination with relevant processes under the CBD.

Outlook and possible elements for a way forward

According to the synthesis of views submitted to the Executive Secretary of the CBD, there are several possible elements for a way forward on the governance of synthetic biology. This would include the need for an open legal framework and transparency to foster awareness by the public and oversight by an informed collection of governments worldwide. Scientific and technological developments in the field of synthetic biology must be reviewed regularly and action taken, particularly if voluntary codes or current regulatory procedures appear insufficient.

In accordance with existing COP decisions, there is agreement that the precautionary approach should be applied to synthetic biology. As such, some are of the view that the environmental and commercial release of organisms resulting from synthetic biology must not occur until procedures and regulatory processes or international regulatory frameworks are in place to ensure the protection of ecological systems.

Many submissions agreed that collaboration with other national and international bodies is needed given the wide-ranging nature and reach of synthetic biology. Of particular importance is the need for a coordinated approach between the CBD and its Protocols, in particular, but not limited to, ensuring strong synergy between the programmes of work on risk assessment and risk management under the Cartagena Protocol and that on synthetic biology under the Convention. The creation of an online platform to facilitate exchange of information on synthetic biology and capacity building would be beneficial in terms of fostering closer collaboration and coordination.

While there is general agreement that existing frameworks can be used as a basis for the risk assessment of organisms developed through synthetic biology, however specific guidelines are still needed to address the additional complexity and risks posed by synthetic biology organisms. As such, it is proposed that there should be review and adaptation of existing frameworks for risk assessment of LMOs. In addition, the need to develop an international framework on synthetic biology that also provides for an assessment of the cultural and socioeconomic impacts was identified.

AHTEG recommendations

The AHTEG on Synthetic Biology also provided additional inputs on a way forward. It recommended, among other things, the establishment of a process to monitor and assess the state of knowledge within the field of synthetic biology on a regular basis, review new information regarding positive and negative impacts and update the proposed operational definition.

The AHTEG also urged Parties to address synthetic biology in a coordinated manner, particularly by tapping into existing processes, such as the AHTEGs on Risk Assessment and Risk Management, and on Socio-economic Considerations under the Cartagena Protocol. Coordination and synergies with other international organisations, the creation of online platforms and tools for sharing information, and the promotion of capacity building and encouragement of cooperation were also highlighted as important steps.

Of note, the AHTEG recommended that mechanisms for clarifying the issue of digital genetic resource information, as it relates to access and benefit-sharing, be set up under the Nagoya Protocol. The AHTEG also called for the assessment of potential gaps in oversight under the Convention and its Protocols with regard to components and products of synthetic biology and the promotion of the full engagement of indigenous peoples and local communities.

Finally, it urged that discussion on the potential benefits and adverse effects of synthetic biology, the development of guidelines, public awareness, communication and education, and ethical considerations, be promoted.

Principles for a regulatory approach

Based on the discussion in this chapter, the following are principles that could apply in order to foster a holistic regulatory approach to synthetic biology:

General

- There should be mandatory regulations applicable to synthetic biology, so as to minimize the potential adverse effects.
- Specific issues for consideration could include a ban on using synthetic biology to manipulate the human genome in any form, due to the ethical issues involved; a prohibition on development of agents for biological warfare (biosecurity considerations); and a moratorium on environmental and commercial release of organisms resulting from synthetic biology until procedures and regulatory processes or international regulatory frameworks are in place.
- The precautionary principle should apply to all aspects of synthetic biology.

Risk assessment

- In order to address the potential adverse effects of synthetic biology, risk assessment becomes necessary.
- This should be a pre-market case-specific assessment that considers direct, indirect, immediate and delayed impacts, and cumulative long-term effects.
- Risk assessment should also take into account risks to human health, and the need to protect public health and worker safety.
- Given that synthetic biology carries many scientific uncertainties, there should always be an acknowledgement of the gaps in scientific knowledge, potential unintentional effects and consideration of uncertainties, including making these known to decision makers.
- If any organism, product or component of synthetic biology is approved, this should be a time-bound approval and reassessment required in case of new information arising.

Other regulatory considerations

- It is critical that socio-economic considerations, including small-scale farming systems and their contribution to biological diversity and ecosystem function, food security and livelihoods, be taken into account.
- In particular, there must be consideration of indigenous peoples and local communities including cultural and ethical aspects.
- The fair and equitable sharing of benefits arising out of the utilization of genetic resources also applies to synthetic biology.
- Any potential damage caused by the organisms, components and products of synthetic biology should be addressed through a liability and redress regime.
- There should be post-market environmental monitoring in order to facilitate risk management and to identify and address any unintentional effects.
- This can be complemented by labelling and traceability measures and ensuring that there are robust detection methods available for the organisms, components and products of synthetic biology.

Complementary issues

- It is important that there is biosafety research to address the gaps in scientific knowledge and uncertainties, *a priori* to commercial release.
- As the technologies are developing rapidly, governments should conduct periodic reviews to ensure that regulations keep pace with technology developments and scientific knowledge.
- Throughout the process, transparency in research and regulation is needed.
- There should be provision of public access to all information regarding decision-making processes, safety testing and products, to ensure open, meaningful and full public participation.
- Governments should also fully consider alternative options to the synthetic biology organism, product or component in question, so as to enable informed decision- making.

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Conclusion

The field of synthetic biology is rapidly evolving and carries great potential to solve present and future societal challenges. Some of the technology advancements are already spurring debates on whether the products should be regulated or not, and to what degree the novel trait is different than what is found in a naturally occurring organism or organisms obtained through classical breeding techniques. The answer to these questions lay in further research and understanding the impact of the new technologies on the natural and technological processes of the cells that are being modified.

Similar to genetically modified organisms, regulation, surveillance and use of synthetic organisms must be coupled to development of models and methods for studying off-target effects, and unintented health and environmental effects. To ensure a sustainable and societal introduction and use of synthetic biology-based products and applications, it is important that biological, environmental, ethical and socio-economic implications are acknowledged. This entails that there is a need for interdisciplinary approaches together with initiatives to raise the awareness of the broader concerns as well as responsibility among those involved with funding, research and development of syntethic biology.

To what extend these products will be regulated, and what regulatory framework that will be applied depends on the outcome of national and international processes and agreements.

The need for capacity building and public awerness of synthetic biology, including both the possibilities, but not the least the challenges are high. Feedback from the participants and lecturers in the courses arranged in 2015 and 2016 showed that many regulatory bodies and systems are not presently in place to ensure a good governance and open public discussions on synthetic biology. This is imperative not only for good governance, but also for public acceptance of research and applications of synthetic biology. If syntheric biology is to play a role as a apart of the solution to challenges like climate change, combating plant and animal diseases and a positive contributor to the bioeconomy, then capacity building of regulatory bodies, academics and in the public are essential to ensure that the technogy reaches its full potential.