Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Scientific Committee on Health and Environmental Risks

SCHER

Scientific Committee on Consumer Safety

SCCS

Final Opinion on

Synthetic Biology III:

Risks to the environment and biodiversity related to synthetic biology and research priorities in the field of synthetic biology

The Scientific Committees adopted this Opinion:

the SCHER at its plenary meeting 27 November 2015, the SCENIHR at its plenary meeting on 3 December 2015 and the SCCS by written procedure on 4 December 2015.

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to new or emerging problems, which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCHER

Opinions on risks related to pollutants in the environmental media and other biological and physical factors or changing physical conditions which may have a negative impact on health and the environment, for example in relation to air quality, waters, waste and soils, as well as on life cycle environmental assessment. It shall also address health and safety issues related to the toxicity and eco-toxicity of biocides.

It may also address questions relating to examination of the toxicity and eco-toxicity of chemical, biochemical and biological compounds whose use may have harmful consequences for human health and the environment. In addition, the Committee will address questions relating to methodological aspect of the assessment of health and environmental risks of chemicals, including mixtures of chemicals, as necessary for providing sound and consistent advice in its own areas of competence as well as to contribute to the relevant issues in close cooperation with other European agencies.

SCHER members

Alena Bartonova, Claire Beausoleil, María José Carroquino, Pim De Voogt, Raquel Duarte-Davidson, Teresa Fernandes, Jadwiga Gzyl, Colin Janssen, Renate Krätke, Jan Linders, Greet Schoeters

SCENIHR

This Committee deals with questions related to emerging or newly identified health and environmental risks and on broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. Examples of potential areas of activity include potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters and electronically controlled home environments), and methodologies for assessing risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases

SCENIHR members

Michelle Epstein, Igor Emri, Philippe Hartemann, Peter Hoet, Norbert Leitgeb, Luis Martínez Martínez, Ana Proykova, Luigi Rizzo, Eduardo Rodriguez-Farré, Lesley Rushton, Konrad Rydzynski, Theodoros Samaras, Emanuela Testai, Theo Vermeire

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.)

SCCS members

Ulrike Bernauer, Qasim Chaudhry, Pieter-Jan Coenraads, Gisela H. Degen, Maria Dusinska, Werner Lilienblum, Elsa Nielsen, Thomas Platzek, Christophe Rousselle, Jan van Benthem

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http://ec.europa.eu/health/scientific_committees/emerging/members_wg/index_en.htm

ABSTRACT

In Opinion I on Synthetic Biology (SynBio), the three Scientific Committees SCHER, SCENIHR and SCCS answered three questions from the European Commission on the scope, definition and identification of the relationship between SynBio and genetic engineering and the possibility of distinguishing the two. The definition reads: 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. In Opinion II, the three Scientific Committees addressed five questions focused on the implications of likely developments in SynBio for humans, animals and the environment and on determining whether existing health and environmental risk assessment practices of the European Union for Genetically Modified Organisms are adequate for SynBio. Additionally, the Scientific Committees were asked to provide suggestions for revised risk assessment methods and risk mitigation procedures including safety locks.

The current Opinion addresses specific risks to the environment from SynBio organisms, processes and products, partly in the context of Decision XI/11 of the Convention of Biodiversity (CBD), identifies major gaps in knowledge to be considered for performing a reliable risk assessment and provides research recommendations resulting from gaps identified. The Scientific Committees confined the scope of their analysis to the foreseeable future, acknowledging that its findings should be reviewed and updated again after several years, depending on the development of the SynBio technology. Outside the scope of the current mandates are specific, thorough analyses of social, governance, ethical and security implications as well as human embryonic research.

Keywords: Synthetic biology; biotechnology; bioengineering; genetic engineering; microbiology; molecular biology; regulatory framework; genetically modified organisms (GMO); risk assessment; risk assessment methodology; risk mitigation; genetic part libraries; minimal cells; designer chassis; protocells and artificial cells; xenobiology; DNA synthesis and genome editing; citizen science; Do-It-Yourself biology.

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EXECUTIVE SUMMARY

In Opinion I on Synthetic Biology (SynBio), the three Scientific Committees (SCs) SCHER, SCENIHR and SCCS answered three questions from the European Commission on the scope, definition and identification of the relationship between SynBio and genetic engineering and the possibility of distinguishing the two. The definition reads: 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. In Opinion II, the SCs addressed five questions focused on the implications of likely developments in SynBio for humans, animals and the environment and on determining whether existing health and environmental risk assessment practices of the European Union for Genetically Modified Organisms (GMOs) are adequate for SynBio. Additionally, the SCs were asked to provide suggestions for revised risk assessment methods and risk mitigation procedures including safety locks.

The current Opinion addresses specific risks to the environment from SynBio organisms, processes and products, partly in the context of Decision XI/11 of the Convention of Biodiversity (CBD), identifies gaps in knowledge that is considered necessary for performing a reliable risk assessment and provides research recommendations resulting from gaps identified. The SCs have confined the scope of their analysis to the foreseeable future, acknowledging that its findings should be reviewed and updated again depending on the progress of SynBio technology. Outside the scope of the current mandates are specific, thorough analyses of social, governance, ethical and security implications of SynBio as well as human embryonic research.

This Opinion addresses questions 9-11 of the SynBio mandate:

Question 9: To review the state of the scientific knowledge concerning specific risks to the environment and synthesise it following the procedure and the requirements mentioned in the Decision XI/11 of the Convention of Biodiversity and include the synthesis in its Opinion.

The SCs analysed how key areas of the application of SynBio may affect the objectives of the CBD. They further analysed impacts on the so-called Aichi Biodiversity Targets for the 2011-2020 period. Bioenergy, agricultural and chemical industry applications of SynBio might drive significant land-use change towards feedstock production which may have negative impacts on biodiversity and conservation, e.g.,

- Increased extraction of biomass from agricultural land or from the natural environment could decrease soil fertility.
- Additional intensification of agriculture with a new end product may lead to effects on soil fertility and to overcome this, additional nutrients may be used.
- Negative impacts could also ensue from accidental releases.
- SynBio produces varieties of organisms, including future de-extincted species and products, and the debate around it could destabilise conservation efforts and diminish support for conservation due to reduced focus on species and habitat preservation.

Risks to the environment were analysed on the basis of Opinion II, key EU Framework projects and pertinent literature. Generic risk factors identified were mostly discussed in relation to impacts on biodiversity and conservation. These risk factors are related to accidental release, persistence of SynBio organisms intended for environmental release, such organisms becoming invasive or disruptive for food webs, transfer of genetic material from vertical gene flow or horizontal gene transfer.

As in Opinions I and II, an analysis of specific risks to the environment was made for each of five novel SynBio developments: 1) Genetic part libraries and methods; 2) Minimal cells and designer chassis; 3) Protocells and artificial cells; 4) Xenobiology: 5) DNA synthesis and genome editing; and 6) Citizen science (e.g., Do-It-Yourself Biology (DIYBio). In general, risks are related to the emergence of new and uncharacterised biological functions, properties and products and the absence of appropriate comparator organisms for the risk assessment means that alternative approaches to risk assessment may be required. With respect to citizen science, the probability of unintentional harm might increase because more people are starting to actively work with biological material outside of conventional laboratory and institutional settings. Genetic firewalls might become necessary for improving containment compared with classical genetic engineering approaches. However, no single technology completely manages all biosafety risks. Many new approaches will be necessary and new forms of biocontainment and additional layers of containment using orthogonal systems will be required to further reduce environmental and health risks. Organisms, whether they are a product of SynBio or not, may not be retrieved once released or escaped into the environment. Risk mitigation is defined as risk reduction measures after deliberate or accidental release of SynBio organisms, components or products and after all biocontainment processes, safety locks and other preventive measures have failed. In specific and high-risk cases, risk mitigation may require a prepared, coordinated, efficient and proportional international response as well as the implementation of WHO International Health Regulation standards including the prior assessment of the necessity for international notification.

Question 10. What are the major gaps in knowledge to be filled for performing a reliable risk assessment in the areas of concern?

The SCs addressed five SynBio research areas and citizen science as key areas of development in Opinion I and II to shed light on gaps of knowledge necessary to perform a reliable risk assessment of the current products and applications of SynBio. Major gaps identified are the lack of information and tools for predicting emergent properties of complex non-standard biological systems and the lack of tools for measurement of the structural differences between the original (natural) and the engineered organism. With respect to protocells, there is little or no information about the behaviour, impact and evolutionary ramifications of interactions of systems consisting of organisms and chemical non-living systems. Hazardous properties of future autonomous, replicating chemical systems, including allergenicity, pathogenicity and biological stability, are unknown. The full mechanistic understanding of underlying principles of semantic containment (e.g., the use of different genetic codes or alternative biochemistries of key informational biopolymers such as nucleic acids or amino acids) that would allow for a reliable prediction of the strength of semantic containment strategies is missing. The use of genome editing methods in a multiplexed fashion allows the simultaneous generation of a large number of variants, the genome-wide modification of organisms and a more accurate and precise change to the genomes of living organisms than those obtained by traditional, targeted genetic modification techniques according to current regulations. It is the scale and speed at which new and complex organisms will be generated and an increase in applications which might create additional challenges for risk assessment.

It is also necessary to establish the degree of risk reduction through the use of genetic firewalls. The methods for submitting genetic modification data and genetic parts information to risk assessors is not yet standardised across EU Member States and internationally, and are largely natural language. Such practices might limit the sophistication of quantitative analyses, data evaluation, efficiency and effectiveness of risk assessment. With respect to citizen scientists, there is a knowledge gap concerning their awareness of and compliance with the established biosafety requirements.

Question 11. SCENIHR, SCHER, and SCCS are requested to provide research recommendations on the main scientific gaps identified. The recommendations should also include methodological guidance on the experimental design and on the requirements of the proposals, in order to ensure data quality and comparability, as well as the usability of the results for risk assessment.

General recommendations

Research on standardised techniques to monitor biocontainment and survival in environments outside the bioreactor and to generate comparative data for use in quantitative biocontainment assessment.

Genetic parts

- Support a) research to characterise the interactions between modified and novel parts, b) development of computational tools to predict emergent new properties of SynBio organisms and their potential failure modes, including biological prediction tools that explicitly incorporate the uncertainty of molecular and genetic information and c) broad dissemination of and training in such tools and knowledge resources.
- Research approaches to streamline and standardise the methods for submitting genetic modification data and genetic parts information, including systems biology models, to risk assessors across EU Member States.
- Develop guidelines for risk assessors on the evaluation of potential emergent properties of genetically engineered systems.
- Research on the use of GMOs with a proven safety record as acceptable comparators for risk assessment so that the baseline state of safe organisms can advance step-by-step with the complexity of new modifications.

Minimal cells and designer chassis

- Research on the introduction of biosafety of modules at the design stage.
- Further fundamental research on quantifying and qualifying the evolutionary change of phenotypes through time is required to understand and predict how these two demands, increased genetic robustness and decreased environmental robustness, can be simultaneously satisfied.

Protocells

- More information is needed to assess the implications, as well as the environmental and evolutionary consequences of a collaborative interaction between non-living protocells and living organisms, including the host range and the specificity of collaborative interactions between protocells and natural cells.
- If protocells become life-like entities, it will be necessary to develop methods to assess the risk of allergenicity, pathogenicity and biological stability.
- More research is necessary to learn and increase knowledge about the ecological and evolutionary role of natural vesicles containing peptides, RNA and DNA.

Xenobiology

- Each individual chemical class of xeno-compounds (e.g., HNA, GNA) should initially be characterised and tested comprehensively (e.g., toxicity and allergenicity), including a risk assessment for emergent properties.

- Establish a methodology to quantitatively and qualitatively characterise xenobiologic organisms with respect to evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases and predation.
- Develop a clear and reliable metric to measure the escape frequency associated with different types of semantic containment.
- Improve the mechanistic understanding of underlying principles of semantic containment to allow for a reliable prediction of the strength of semantic containment strategies.

Citizen science

The SCs recommend the development of strategies to further increase and maintain the compliance of citizen scientists with harmonised European biosafety rules and codes of ethics, including collaboration with acknowledged institutions and training.

Additional research recommendations

For the improvement of risk assessment, additional recommendations were derived from the analysis of impacts on biodiversity and conservation and specific risks to the environment, including research on:

- Impacts from accidental or intentional introduction of SynBio organisms into the environment with emphasis on the effects on habitats, food webs and biodiversity.
- The difference in physiology of natural and synthetic organisms.
- Vertical or horizontal gene flow.
- Survival, persistence, ecological fitness and rate of evolutionary change.
- ”de-extinction” and the debate around it.
- Containment strategies to prevent unintentional release of or exposure to organisms resulting from SynBio techniques.
- The environmental performance of SynBio processes and products, considering the full product life cycle.
- An emerging technology that uses similar techniques to the ones that are commonly applied in genome editing for SynBio applications are the so-called "gene drives". However, for the purposes of this Opinion, gene drives are not considered as falling under the definition of SynBio. While the methods used are related, gene drives aim at modifying the genetic composition of populations, not of individual organisms: an analysis of the risks and implications of “gene drives” is therefore outside the scope of this Opinion. Nevertheless, the increasing use of gene drive technology would certainly require a similar in-depth analysis, including a detailed assessment of its implications for risk assessment methodology and its potential impact on biodiversity and the environment.

Prioritisation of impact assessments can be based on prior knowledge available.

1. BACKGROUND

This Opinion is the third in a series of three Opinions on Synthetic Biology (SynBio) responding to questions from the European Commission (Annex I). The overall, legal and scientific background underlying these questions from the Commission was discussed in the first Opinion (2014) and methodological and safety aspects were discussed in the second Opinion (2015). Abstracts of Opinion I and Opinion II are included in Annexes II and III, respectively.

1.1.General introduction

SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms. Synthetic biologists use engineering principles and re-design existing systems to better understand life processes. In addition, the objective is to generate and assemble functional modular components for the development of novel applications and processes such as synthetic life, cells or genomes. SynBio processes offer novel opportunities for the creation of new industries with profound economic implications for the European Union (EU) and other major economies. Just as advances in synthetic chemistry had a major impact on the shaping of modern societal and economic structures in the 19th and 20th centuries, SynBio promises substantial benefits for health, the environment, resource management and the economy. In addition to the promised benefits of SynBio, there are scientific uncertainties associated with the development of synthetic life, cells or genomes and their potential impact on the environment, the conservation and sustainable use of biological diversity and human health. A precautionary approach in accordance with domestic legislation and other relevant international obligations is required to prevent the reduction or loss of biological diversity posed by organisms, components and products generated by SynBio.

1.2.Legal background

In December 2008, an EU Member State expert Working Group was established to analyse a list of new techniques which supposedly result in genetically modified organisms (GMOs) as defined under Directive 2001/18/EC on the deliberate release of GMOs and Directive 2009/41/EC on contained use of GM microorganisms (GMMs). Although most of the techniques analysed by the New Techniques Working Group (NTWG, 2012 New techniques working group, Final Report) were focused on the direct implications on plant breeding, synthetic genomics as a field within SynBio that may include techniques of genetic modification was also considered. The Report from this Working Group was finalised in January 2012 (NTWG, 2012) and the main conclusion was that synthetic genomics / SynBio is a fast-evolving field that differs from previous gene modification techniques. Furthermore, the NT Working Group was uncertain whether Directives 2009/41/EC and 2001/18/EC (see Section Annex V from the European GMO regulatory framework) were the appropriate legislation to cover synthetic genomics and SynBio. The SynBio WG was established with the mandate to address these uncertainties and to explore the implications of SynBio, including but not limited to synthetic genomics and related technologies.

2. TERMS OF REFERENCE

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) was requested[1] to answer the following questions through a joint Opinion in association with SCHER and SCCS and, if relevant, other European Community bodies e.g., the European Environmental Agency (EEA) and the European Food Safety Agency (EFSA).

According to Terms of Reference, The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) was requested to answer a set of 11 questions from the European Commission on SynBio (see annex I) through a joint Opinion in association with SCHER and SCCS and, if relevant, other European Community bodies e.g., the European Environmental Agency (EEA) and the European Food Safety Agency (EFSA). Questions 1-8 were answered in SynBio Opinions I and II. (SCENIHR, SCCS, SCHER, 2014 and 2015). Questions 9 through 11 are addressed in the present Opinion. The abstracts of SynBio I and SynBio II Opinions are attached as Annex II to the present Opinion. Although security issues[2] concerning SynBio are also important, the terms of reference pertain exclusively to safety and, thus, security issues will not be addressed in any of the three Opinions. In addition, the SCs did not deliberately address human embryonic research because it is outside of the scope of the mandate.

Questions 9-11 of the Terms of Reference

9. The SCENIHR, SCHER, SCCS are asked to review the state of the scientific knowledge concerning specific risks to the environment and synthesise it following the procedure and the requirements mentioned in the Decision XI/11 of the Convention of Biodiversity (COP Decision XI/11) and include the synthesis in its Opinion.

10. What are the major gaps in knowledge to be filled for performing a reliable risk assessment in the areas of concern?

11. SCENIHR, SCHER, and SCCS are requested to provide research recommendations on the main scientific gaps identified. The recommendations should also include methodological guidance on the experimental design and on the requirements of the proposals, in order to ensure data quality and comparability, as well as the usability of the results for risk assessment.[2]

3. SCIENTIFIC RATIONALE

3.1. Methodology

The aim of this work was to identify the nature and scope of activities related to the subject of SynBio. Information was primarily obtained from reports published in international peer-reviewed scientific journals in the English language. Additional sources of information were considered, including web-based information retrieval and documents from governmental bodies, authorities and non-governmental organisations. To facilitate the task of the Committee, the EC contracted 3 searches of the published literature. The first covered SynBio literature published from 2000 up to the beginning of 2013, the second up to early 2014 and the third covered papers published up to and including February 2015. In addition, a search was conducted of publications by governmental bodies relating to the regulation of GMOs and SynBio. The searches yielded approximately 800 publications. Relevant documents published before March 1st 2015, the

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[1] European Commission (2013) Request for a joint scientific opinion on Synthetic Biology. Brussels.

[2] Biosafety principles and practices aim at preventing the unintentional release of pathogens and/or toxins ("keeping bad bugs from people"); Biosecurity seeks to prevent the intentional release of pathogens and/or toxins ("keeping bad people from bugs"); European Parliamentary Technology Assessment (2011). EPTA Briefing Notes 1.

closing date for data considered for this Opinion, were identified and critically examined. Not all identified studies were included in the Opinion. The main task was to evaluate and assess the articles, their relevance to the topic and the scientific weight given to each of them. Only studies that were considered relevant for the task were included and commented upon in the Opinion. In some areas where the literature is particularly scarce, an explanation is provided for clarification. Detailed criteria for selecting studies were published in the SCENIHR Memorandum "Use of the scientific literature for human health risk assessment purposes, weighing of evidence and expression of uncertainty" (SCENIHR, 2012).

3.2. To review the state of the scientific knowledge concerning specific risks to the environment and synthesise it following the procedure and the requirements mentioned in the Decision XI/11 of the CBD and include the synthesis in its Opinion

3.2.1. Introduction

In Opinions I and II, the SCs addressed the current knowledge of SynBio-related risks to the environment and health. In this section, risks to the environment in the context of COP Decision XI/11 of the CBD are elaborated with focus on the main research areas in SynBio (see Annex IV). First, the key issues in COP Decision XI/11 are explained, followed by an overview of positive and negative potential impacts. Next follows an analysis of specific risks to the environment with reference to Opinion II, key EU Framework projects and pertinent literature.

3.2.2. Key issues in the Decision XI/11 of the CBD that affect SynBio

The key focus of this section is to address how SynBio may affect the objectives of the Convention on Biological Diversity, particularly by addressing any activities or processes that may lead to loss of biodiversity and ensure the implementation of actions that effectively reduce the rate of, halt or reverse the loss of biodiversity. The main relevant issues in this area are addressed in the Strategic Plan for Biodiversity (2011-2020), complemented by national biodiversity strategies and action plans.

Particularly COP Decision XI/11 with reference to IX/29 (Opinion I; section 3.3.2.8 and 3.3.2.9), §11 and §12, refers to the need to identify new and emerging issues related to the conservation and sustainable use of biodiversity. Criteria that will be used for identifying new and emerging issues related to conservation and sustainable use of biodiversity are laid down and include particular considerations on relevance, evidence, urgency, potential magnitude of impact on biodiversity, human well-being and/or services, geographic coverage, limitation/mitigation measures. To evaluate how these criteria apply to SynBio (COP Decision XI/11)*, the Conference of the Parties* requested the Executive Secretary of the Convention of Biodiversity (CBD) to compile and synthesise relevant information on components, organisms and products obtained by the use of SynBio techniques that may have impacts on the conservation and sustainable use of biological diversity and associated social, economic and cultural considerations (see document UNEP/CBD/COP/12/INF/11). In addition, this should address any possible gaps and overlaps with the applicable provisions of the CBD, its Protocols and other relevant agreements related to components, organisms and products obtained by the use of SynBio techniques (see document UNEP/CBD/COP/12/INF/12). After both documents (UNEP/CBD/COP/12/INF/11 and UNEP/CBD/COP/12/INF/12) were subject to peer review and discussed during the eighteenth meeting of the Subsidiary Body on Scientific Technical and Technological Advice (SBSTTA) (June 2014), the documents were made available to the twelfth meeting of the Conference of the Parties to the Convention on Biological Diversity in October 2014.

During its eighteenth meeting, the SBSTTA recognised that 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 are of relevance to the Convention. However, it also concluded that there is currently insufficient information available to finalise an analysis, using the criteria set out in §12 of Decision IX/29. Taking this into account, the Conference of the Parties to the CBD maintained its decision to take a precautionary approach, and it now awaits the completion of a robust analysis (Decision XII/24 of CBD). To this end, the executive secretary of the CBD will continue to compile relevant information submitted by Parties, governments, relevant organisations and other stakeholders. In addition, an Ad Hoc Technical Expert Group was established on the basis of the terms of reference as outlined in Decision XII/24 of CBD and met for the first time in 21 September 2015 (CBD, 2015b).

In this process, the main focus has been set on effective risk assessment and management procedures for regulating environmental release of any organisms, components or products resulting from SynBio applications as well as scientific assessments regarding potential effects on the conservation and sustainable use of biodiversity. Other issues are also addressed such as food security and socio-economic considerations, funding for research into SynBio risk assessment methodologies and promotion of interdisciplinary research that includes related socio-economic considerations. Appropriate risk assessment should be in place prior to any field trials for organisms, components or products resulting from SynBio applications.

3.2.3. Potential impacts of SynBio applications on conservation and sustainable use of biodiversity

The text in this section highlights the key areas of application of SynBio that may impact biodiversity and conservation. These include potential positive and negative impacts as highlighted in UNEP/CBD/COP/12/INF/11.

Bioenergy applications of SynBio applied on a large scale: SynBio applications in the area of Bioenergy could reduce global dependence on fossil fuels and reduce harmful emissions (PCSBI 2010).

- SynBio tools may be used in designing "next generation" biofuels that will overcome challenges of "first generation" biofuels made from food crops (Webb & Coates 2012). SynBio offers the potential to overcome some perplexing technical barriers for the production of second-generation biofuels from non-food crops and waste. Three areas of high relevance are consolidated bioprocesses (CBP) (e.g., Bokinsky *et al.*, 2011), micro- (Reijnders *et al.*, 2014) and macro-algae (van Hal *et al.*, 2014) for biofuels and fermentation of industrial waste gases (Bomgardner, 2012). In CBP, both biomass-degrading and biofuel-producing capabilities are incorporated into a single organism: this may be the ultimate low-cost configuration for cellulose hydrolysis and fermentation (US DoE, 2006). The use of algae for biofuels production relieves pressure on land, but natural microbial strains are not optimised for industrial production (Raman *et al.*, 2014). Similarly, industrial waste gas fermentation removes the need for biomass, but this relies heavily on genetic modification – it is ripe for SynBio research.
- Use of biomass as feedstock in SynBio processes may be an environmentally beneficial shift from non-renewable resources (Erickson *et al.,* 2011; Georgianna & Mayfield 2012).
- SynBio bioenergy applications could lead to increased extraction of biomass from agricultural land, which may decrease soil fertility and would potentially affect nutrient use and management (ICSWGSB 2011; Fixen 2007).

- Increased demand for biomass could lead to displacement of local sustainable uses and lead to environmental harm in tropical and sub-tropical communities (ETC 2010; FOE *et al.,* 2012; FOE 2010).
- If SynBio techniques open up new sources of energy such as algae and seaweed, increased demand might encroach on traditional uses of these resources (ETC 2013).
- The accidental release of organisms resulting from SynBio techniques for bioenergy production could have a negative impact on biodiversity and conservation (section 3.1.5).
- Bioenergy production and use have the dual goal of increasing energy security and mitigating climate change. Biofuels policies in Europe centre on the Renewable Energy Directive and the partial replacement of fossil fuels with biofuels to help meet emissions targets.

Environmental applications of SynBio

- Microorganisms resulting from SynBio techniques may be used in the degradation of contaminants, leading to a more 'environmentally sound' approach to bioremediation (Kirby 2010).
- Microorganisms resulting from SynBio techniques may be used as biosensors, helping to identify areas contaminated with specific pollutants (French *et al.,* 2011).
- The deliberate release into the environment of microorganisms obtained by the use of SynBio techniques could potentially have negative impacts on biodiversity and conservation (section 3.1.4).

Wildlife-targeted applications of SynBio

- It has been suggested that SynBio applications should in the long-term be used to restore extinct species ("de-extinction"), and this has been suggested as possibly leading to the restoration of ecological richness (Church 2013; Redford *et al.,* 2013). It has been proposed that de-extinction could provide a new paradigm for biodiversity advocacy, based on pro-active action, rather than post-effect activity (Brand 2013; Redford 2013).
- It has, on the other hand, been suggested that de-extinction research may have a destabilising effect on conservation, potentially resulting in species loss, due to potentially reduced focus on species and habitat preservation (Temple 2013). For example, proposed SynBio approaches might move voluntary and statutory stakeholders away from addressing underlying causes for biodiversity loss (Ehrenfeld 2013; Ehrlich 2013, Redford *et al.,* 2013). Similarly, support for *in situ* conservation might be reduced, with impacts on support for existing protected areas potentially increasing (Redford *et al.,* 2013). The same authors describe the potentially reduced willingness to conserve endangered species as a "moral hazard" of de-extinction research.
- SynBio applications might help to identify and treat wildlife diseases (Allendorf *et al.,* 2010), as well as target threats to wildlife, such as disease vectors (Weber & Fussenegger, 2012).

Agricultural applications of SynBio

- The use of synthetic organisms in the agricultural production sectors might foster ''sustainable intensification'' and ''land sparing'', leading to reduced land conversion and increased protection of wild habitats (Redford *et al.,* 2013).
- Reduced use of chemical pesticides and fertilisers enabled by the use of genetically modified crops could have positive ecological impacts (PCSBI 2010).
- Industrial uses of SynBio might drive significant land-use change towards feedstock production, which could have beneficial or negative impacts on biodiversity and conservation (Erickson *et al.,* 2011; Redford *et al.,* 2013).

Applications of SynBio to replace natural materials

- Molecules produced through SynBio could enable conservation of plants and animals currently unsustainably harvested from the wild or through unsustainable cultivation (BIO 2012).

Applications of SynBio to replace materials made with synthetic chemistry

- SynBio alternatives for chemical products and industrial processes could lead to decreased use of non-renewable resources and less environmentally harmful manufacturing processes (Garfinkel & Friedman 2010).
- The increased use of SynBio-based production processes could promote the transition to sustainable production and consumption, which might protect biodiversity (Redford *et al.,* 2013).
- SynBio alternatives for chemical products and industrial processes might not actually be more sustainable than traditional products; this has, e.g., been argued in the case of current bioplastics (ETC 2010, Schmidt 2012).
- Industrial uses of SynBio might drive significant land-use change towards feedstock production, which could have beneficial or negative impacts on biodiversity and conservation (Erickson *et al.,* 2011; Redford *et al.,* 2013).
- The transition to a bioeconomy envisages a gradual replacement of fossil fuels and petrochemicals with bio-based equivalents (using sugar bio-based carbon compounds as feedstock instead of oil or gas) (US DoE 2004). The basis is that bio-based equivalents should produce fewer negative environmental impacts and can be used by countries to meet their emissions reduction targets in line with the goals of the Copenhagen Accord, whilst also protecting biodiversity.
- Based on twelve extremely important industrial materials, Saygin *et al.* (2014) estimated significant CO_2 emissions savings of some bio-based materials compared to their petrochemical equivalents. These savings translate to an average of 2.5 ± 1.6 tonnes less CO_2 emitted per tonne bio-based material produced confirming earlier findings by Weiss *et al.* (2012), and consistent with Hermann *et al.* (2007).
- The environmental performance of bio-based materials should remain a research focus due to a host of future uncertainties e.g., fossil fuel prices, sugar prices, individual differences in emissions reductions of bio-based materials and indirect land use change (ILUC) developments.

In decision X/2 of the tenth meeting of the Conference of the Parties, held from 18 to 29 October 2010, in Nagoya, Aichi Prefecture, Japan, a revised and updated Strategic Plan for Biodiversity, including the Aichi Biodiversity Targets, for the 2011-2020 period was adopted. Table 1 highlights the potential SynBio impacts on reaching the Aichi Biodiversity Targets. The timescales mentioned in the Aichi targets may be too ambitious. The predictions made by SCs do not go beyond 2025 (10 years ahead).

Table 1: Potential impacts of SynBio on reaching the Aichi targets

Strategic Goal A: Address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
1. By 2020, at the latest, people are aware of the values of biodiversity and the steps they can take to conserve and use it sustainably.	Positive: nature provides a great amount of not yet discovered useful genetic parts. Contribution to sustainable use of biodiversity.	None	None	Negative: artificial diversity could lead to lack of perceived value of natural biodiversity.	DNA Synthesis (in connection with DNA sequencing) in combination with potential species de-extinction could undermine conservation efforts; consequently decreasing the perceived value of true biodiversity. Genome editing = engineered diversity at intra-species level may reduce incentive for maintaining genetic diversity of natural organisms.	Citizen science, in its role as engaging lay people with science and biology, could help to increase appreciation for natural biodiversity and its value.
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
2. By 2020, at the latest, biodiversity values have been integrated into national and local development and poverty reduction strategies and planning processes and are being incorporated into national accounting, as appropriate, and reporting systems.	None	None	None	None	None	None
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
3. By 2020, at the latest, incentives, including subsidies, harmful to biodiversity are eliminated, phased out or reformed to minimise or avoid negative impacts, and positive incentives for the conservation and sustainable use of biodiversity are	None	None	None	None	None	None

developed and applied, consistent and in harmony with the Convention and other relevant international obligations, taking into account national socio economic conditions.						

Strategic Goal B: Reduce the direct pressures on biodiversity and promote sustainable use

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
4. By 2020, at the latest, governments, business and stakeholders at all levels have taken steps to achieve or have implemented plans for sustainable production and consumption and have kept the impacts of use of natural resources well within safe ecological limits.	Potentially, "green" production methods based on SynBio could lead to reduced consumption of non-renewable resources (esp. oil), but also risk of increased burden on the natural environment and conflict with keeping impact within safe ecological limits. While production of certain chemicals may be made more efficient, increased demand for raw material (sugar) could have a detrimental impact on biodiversity	None	None	None	None	Citizen science could help to promote sustainable production and consumption.
5. By 2020, the rate of loss of all natural habitats, including forests, is at least halved and where feasible brought close to zero, and degradation and fragmentation are significantly reduced.						

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
6. By 2020 all fish and invertebrate stocks and aquatic plants are managed and harvested sustainably, legally and by applying ecosystem-based approaches, so that overfishing is avoided, recovery plans and measures are in place for all depleted species, fisheries have no significant adverse impacts on threatened species and vulnerable ecosystems and the impacts of fisheries on stocks, species and ecosystems are within safe ecological limits.	None	None	None	None	None	None
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
7. By 2020, areas under agriculture, aquaculture and forestry are managed sustainably, ensuring conservation of biodiversity.	Positive: Genetically modified crops produced by SynBio could lead to decreases in pesticide or fertiliser use, as seen or expected for some established GMO crops (e.g., Bt strains). Negative: Concerns have been raised about the effect of such genetically modified crops on the biodiversity in agro-ecosystems, e.g., toxicity to non-target species. This concern could potentially also apply to the next generation of SynBio crops.	None	None	None	None	None

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
8. By 2020, pollution, including from excess nutrients, has been brought to levels that are not detrimental to ecosystem function and biodiversity.	Industrial processes that produce pollution may be superseded by more SynBio based environmentally friendly replacements.	None	None	None	None	None
9. By 2020, invasive alien species and pathways are identified and prioritised, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment.	Neutral in the medium term: potential biocontrol strategies based on SynBio are too immature to consider using them. Potentially negative beyond 2020: synthetically modified species could become invasive.	None	None	Forms of life not known from nature could on the one hand be considered to increase biodiversity (if one accepts the idea that organisms that are not linked to the common evolutionary tree are contributing to biodiversity), but could also lead to the establishment of novel invasive species.	None	None
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
10. By 2015, the multiple anthropogenic pressures on coral reefs and other vulnerable ecosystems impacted by climate change or ocean acidification are minimised, so as to maintain their integrity and functioning.	2015! None	2015! None	2015! None	2015! None	2015! None	2015! None

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Strategic Goal C: To improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity						
Aichi TARGETS	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
11. By 2020, at least 17 per cent of terrestrial and inland water, and 10 per cent of coastal and marine areas, especially areas of particular importance for biodiversity and ecosystem services, are conserved through effectively and equitably managed, ecologically representative and well connected systems of protected areas and other effective area-based conservation measures, and integrated into the wider landscapes and seascapes.	None	None	None	None	None	None
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
12. By 2020 the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained.	None	None	None	None	None	None
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
13. By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimising genetic erosion and safeguarding their genetic diversity.	Positive: SynBio could result in a renewed appreciation of the value of genetic diversity of cultivated plants and farmed and domesticated animals, as a source of valuable building blocks for genetic engineering approaches. Negative: The ability of designing and producing improved plant varieties based on genome sequence	None	None	None	Rather negative, with the option to re-synthesise any genome from scratch, the pressure to maintain landraces and wild relatives will become more and more reduced.	None

	data could reduce the focus on conserving old land races and the need to preserve wild relatives, once they have been sequenced.					

Strategic Goal D: Enhance the benefits to all from biodiversity and ecosystem services						
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
14. By 2020, ecosystems that provide essential services, including services related to water, and that contribute to health, livelihoods and well-being, are restored and safeguarded, taking into account the needs of women, indigenous and local communities, and the poor and vulnerable.	Ranging from positive to negative. Biosensors, for example, could help people in poor countries to test the quality of the water. But in general the design goals in SynBio are almost exclusively driven by developed countries, financial and intellectual elites, and so far very little attention has been paid to the interests of the marginalised communities, and the poor and vulnerable.	Maybe as a chassis for biosensors. See left field.	None	None	None	DIYBio already helps to empower women, indigenous and local communities, and the poor and vulnerable to use (synthetic) biology for their own needs. It is, however, not clear if this will contribute to the restoration and safeguarding of essential ecosystem services.

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
15. By 2020, ecosystem resilience and the contribution of biodiversity to carbon stocks has been enhanced, through conservation and restoration, including restoration of at least 15 per cent of degraded ecosystems, thereby contributing to climate change mitigation and adaptation and to combating desertification.	Positive: SynBio organisms could lead to more resilient (salt/draught resistant) agro-ecosystems that could contribute to reversing desertification and support a higher level of biodiversity, e.g., due to reduced use of pesticides. Negative: increased drain on natural resources to generate feedstock for SynBio.	None	None	None	None	None
16. By 2015, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation is in force and operational, consistent with national legislation.	None	None	None	None	None. However, the Nagoya Protocol does not explicitly define the exchange of genomic data, e.g., sequenced in one country, sent by electronic means (not physically) and then synthesised in another country. So, DNA sequencing and synthesis could provide a loophole to the Nagoya protocol.	None

Strategic Goal E: Enhance implementation through participatory planning, knowledge management and capacity building

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
17. By 2015 each Party has developed, adopted as a policy instrument and commenced implementing an effective, participatory and updated national biodiversity strategy and action plan.	None	None	None	None	None	None
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
18. By 2020, the traditional knowledge, innovations and practices of indigenous and local communities relevant for the conservation and sustainable use of biodiversity, and their customary use of biological resources, are respected, subject to national legislation and relevant international obligations, and fully integrated and reflected in the implementation of the Convention with the full and effective participation of indigenous and local communities, at all relevant levels.	Negative: see targets 14 and 16. Also, SynBio methods (genetic parts, DNA synthesis) might decrease the value of customary use of biological resources, threatening traditional practices and excluding indigenous and local communities from the exploitation of biological diversity.	None	None	None	Negative: see target 16. Also, SynBio methods (genetic parts, DNA synthesis) might decrease the value of customary uses of biological resources, threatening traditional practices and excluding indigenous and local communities from the exploitation of biological diversity.	DIYBio in developing countries could help to increase awareness of traditional knowledge and local biological resources.
Aichi TARGET	**Genetic parts**	**Minimal cells and designer chassis**	**Protocells**	**Xenobiology**	**DNA synthesis**	**Citizen science**
19. By 2020, knowledge, the science base and technologies relating to biodiversity, its values, functioning, status and trends, and the consequences of its loss, are improved, widely shared and transferred, and applied.	None, possibly positive: scientific insights based on attempts to engineer organisms by SynBio could contribute to a better understanding of natural systems. Research into containment strategies for SynBio organisms will lead to more fundamental insights into population genetics, population dynamics, evolution and ecology. This target is not just about improving knowledge, but also about sharing, transferring, and applying knowledge. It is thus an appropriate target for considering intellectual property in the context of SynBio.					

Aichi TARGET	Genetic parts	Minimal cells and designer chassis	Protocells	Xenobiology	DNA synthesis	Citizen science
20. By 2020, at the latest, the mobilisation of financial resources for effectively implementing the Strategic Plan for Biodiversity 2011-2020 from all sources, and in accordance with the consolidated and agreed process in the Strategy for Resource Mobilisation, should increase substantially from the current levels. This target will be subject to changes contingent to resource needs assessments to be developed and reported by Parties.	None	None	None	None	None	None

3.2.4. Specific risks to the environment per research area

General issues

The following events were considered in the literature. These would need a more thorough analysis of the probability at which they can occur and the impacts these may have. The order of the generic risks is not in order of priority.

- Accidental release of SynBio organisms engineered for contained use may lead to their survival and propagation in the environment (Garfinkel and Friedman, 2010; Lorenzo, 2010; RAE 2009; Snow and Smith, 2012; Dana *et al.*, 2012).
- Accidental release could affect water/wastewater treatment processes (specifically biological processes) through the interaction with indigenous microorganisms (Unnithan *et al*., 2014; Guo *et al*., 2014) as well as they may be undertaken to unpredictable genetic changes/transformations (e.g., mutants formation, antibiotic resistance transfer) in chemical oxidation/disinfection (based water/wastewater treatment plants (Dunlop *et al.*, 2015; Luddeke *et al*., 2015).
- Persistence of an organism designed for environmental release. (Anderson *et al.*, 2012; Pauwels *et al.,* 2012).
- Organisms resulting from SynBio techniques could become invasive or disrupt food webs (Redford *et al.*, 2013; Snow and Smith, 2012; Wright *et al.*, 2013).
- Transfer of DNA from vertical gene flow or horizontal gene transfer (König *et al.,* 2013; Wright *et al.*, 2013).
- Potential impacts on biodiversity and ecosystems from ”de-extinction” (Donlan, 2014; Seddon *et al.*, 2014).

Genetic parts

SynBio library construction and parts characterisation may increase the frequency of use of uncharacterised components, and/or the diversity of biological functions. The function of these systems may be "emergent," i.e. they arise from the interactions of the parts with each other. Emergent functions may include conditional, time-varying and non-linear (non-proportional) behaviours (Guet *et al.*, 2002). The current Directives 2001/18/EC and 2009/41/EC for risk assessment consider these emergent properties by requiring an assessment of the proposed or realised GMM/GMO, in addition to an assessment of the properties of component parts. Notably, the emergent properties may present new challenges in predicting or testing for risks and in the identification of appropriate comparator organisms.

Minimal cells and designer chassis

The four primary biosafety considerations with chassis cells are (Dana *et al.*, 2012):

- Survival of synthetic organisms in receiving environments.
- Gene transfer.
- Interactions between synthetic and natural organisms.
- Adaptation of synthetic organisms to new ecological niches.

Much depends on the ability of a chassis organism to survive in the environment and to exchange genetic material with other organisms within it. For biotechnology applications, reducing the genomes of *Escherichia coli* (E. *coli*) and other minimal risk (BSL-1) biotechnology workhorses seems most useful (Jewett & Forster, 2010). On the other hand, minimal genomes may not constitute the best chassis, because robust and rapid growth and access to multiple pathways seem to benefit from larger genomes.

In many cases, commonly used chassis organisms, often derived by a process of laboratory "domestication" from wild-type bacteria and yeasts (e.g., Saccharomyces), are generally safe – their genome is already significantly reduced during the process of domestication, removing, e.g., a variety of pathogenicity factors and introducing useful fragilities to the system to further reduce escape potential. Of great significance to biosafety is the fact that, with a highly reduced genome, SynBio-based minimal cells will be restricted to a very narrow ecological niche (Schmidt *et al.*, 2009), and are less likely to survive for long periods in the event of accidents releasing them to the environment, typically wastewater treatment systems and soil.

While confinement to a small ecological niche is likely when considering the continuous existence of an independent organism, other evolutionary routes could lead to the establishment of an endosymbiontic relationship with another organism and eventually the establishment of an organelle (see e.g., Ochoa 2014, McFadden 2001). It is unclear if a small genome or cell size would favour the uptake of the cell by another bigger cell, but it could indeed increase the chance for the evolution of a new endosymbiont/organelle. Research into possible ongoing endosymbiontic processes could help to shed more light on the matter (Okamoto and Inouye 2005).

Another point of reference is the very large or "giant" virus (Claverie *et al.,* 2006). Recent years have seen the discovery (La Scola *et al.,* 2003) of a large virus with genomes (>1Mbp) larger than even the smallest genomes of free, living cells (e.g., Mycoplasma species can have only 0.58 Mbp). Little is known about the evolution of the large virus, but it cannot be ruled out that they derive from small cells. Mimivirus, for example, still owns a much more complete set of translation-associated genes than non-giant virus. Some researchers have speculated that the Mimivirus may have evolved from a free-living cell (Raoult *et al.,* 2004). Future research will need to explore the origin of this large virus and if minimal cells have any reasonable chance of "downgrading" themselves to a viral existence.

Protocells

Currently, protocells are non-living vesicles and will likely be confined to the laboratory for the short- to medium-term. Although the objective is for such cells to replicate, this is not yet possible. Therefore, dispersion is not possible because of the lack of cell viability. Risks related to protocell research are no higher than the risks in biological and chemistry laboratories because the current state-of-the-art research does not create novel, viable artificial cells. In the future, exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Although protocells are not alive, they can be engineered to intimately interact with living cells and enhance overall system functionality (Lentini *et al*., 2014). Thus, novel biological functions can be designed without altering the DNA of these target organisms. If autonomous artificial cells are created in the future, the genetic information that controls internal functioning might mutate or be horizontally transferred. Thus, a population of protocells with different genetic information could undergo selection and new protocells could arise (Bedau *et al.*, 2009).

Xenobiology

The use of non-standard biochemical systems in living cells, e.g., xenonucleic acid XNA, alternative base pairs, etc., has implications for risk assessment and biosafety. New variants must be tested for risk to human health or to the environment, and the xenobiological systems may be engineered to allow for improved biocontainment, e.g., the so-called 'genetic firewall' that aims to avoid the exchange of genetic material through horizontal gene transfer or sexual reproduction between the xenobiology and natural organisms. The assumption is that xeno-systems would not survive after accidental release due to their custom-made auxotrophies.

DNA synthesis and genome editing

The new technologies for DNA synthesis and genome editing such as TALEN, CRISPR (Sander et al., 2014; Zetsche et al., 2015) and MAGE (Gallagher et al., 2014; Kang et al., 2015) accelerate genetic modification and increase the range and number of modifications that are easily possible. The increased speed of modifications might pose challenges to risk assessment, while not in itself creating new risks.

An emerging technology that uses similar techniques to the ones that are commonly applied in genome editing for SynBio applications are the so-called "gene drives" (Esvelt et al. 2014; Oye et al., 2014; Gantz and Bier, 2015). However, for the purposes of this Opinion, gene drives are not considered as falling under the definition of SynBio, i.e. "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms". While the methods used are related, gene drives aim at modifying the genetic composition of populations, not of individual organisms; an analysis of the risks and implications of "gene drives" is therefore outside the scope of this Opinion. Nevertheless, the increasing use of gene drive technology would certainly require a similar in-depth analysis, including a detailed assessment of its implications for risk assessment methodology and its potential impact on biodiversity and the environment.

Citizen science

While the hazard remains the same, e.g., infection with pathogenic organisms, the probability of unintentional harm might increase, because more people are starting to actively work with biological material outside of conventional laboratory and institutional settings. However, as long as the citizen science community is well informed and adequate safety measures are implemented (equivalent to those implemented in the traditional professional community), the overall additional risk would be minimal.

3.2.5. Prevention of SynBio adverse effects on the environment

An important task of a safety discussion is to explore how SynBio itself may contribute towards overcoming existing and possible future biosafety problems by contributing to the design of safer biosystems, for example: A) Designing less competitive organisms by changing metabolic pathways; B) Replacing metabolic pathways with others that have an in-built dependency on external biochemicals; C) Designing evolutionary robust biological circuits; D) Using biological systems based on an alternative biochemical structure to avoid e.g., gene flow to and from wild species; E) Designing protocells that lack key features of living entities, such as growth or replication (Schmidt, 2009). Gressel *et al.* (2013), for instance, discuss the environmental risk of spills of genetically modified microalgae used for biofuels production by physical containment and by genetically precluding the algae from replicating and competing in nature by introducing genes which severely decrease their fitness in natural ecosystems. Silencing or loss of such traits can be prevented by coupling them with a selectable trait such as herbicide resistance.

In Opinion II (Final Opinion on synthetic biology II, 2015), the SCs stressed that currently available safety locks used in genetic engineering are not yet sufficiently reliable for SynBio. For instance, genetic safeguards such as auxotrophy and kill switches are not sufficiently reliable/robust for field release of engineered bacteria because of mutation and positive selection pressure for mutants that may lead them to escape safeguards. Notably, SynBio approaches that provide additional safety levels, such as genetic firewalls, may improve containment compared with classical genetic engineering. However, no single technology solves all biosafety risks, and many new approaches and combinations of existing and upcoming new strategies will be necessary.

Coming up with a blueprint of a general strategy for designing inherently safe applications of SynBio is demanding because of the stochastic and probabilistic character of the underlying biochemical SynBio processes and the incomplete characterisation of the parts and chassis used in SynBio engineering, as well as their potential interactions. General biocontainment approaches are based on 1) physical containment, 2) inhibition of uptake, 3) incorrect translation, 4) inability to replicate, 5) absence of host immunity and 5) endogenous toxicity. The SCs recommended a clear strategy for the analysis, development, testing and prototyping of applications based on new forms of biocontainment and additional layers of containment using orthogonal systems.

3.2.6. Mitigation of SynBio adverse effects on the environment

Mitigation is defined by the SCs as risk reduction measures that can be taken after deliberate or accidental release of SynBio organisms, components or products and when all biocontainment processes, safety locks and other preventive measures have failed. It is widely asserted that organisms, resulting from SynBio techniques or not, may not be retrieved once released (Dana *et al.,* 2012; Snow and Smith, 2012; CBD, 2015a).

For the prevention of a biological incident of any type, the main goal of contingency planning should be to mitigate an event whether it is deliberate, accidental, or a naturally occurring release, which may be difficult to distinguish at first. In specific and in high-risk cases, a prepared, efficient and proportional international response may limit the size and scope of such releases as well as the implementation of IHR standards (international health regulations; WHO, 2005), including the prior assessment of the necessity for international notification (Gronvall, 2015).

3.3. Major gaps in knowledge to be considered for performing a reliable risk assessment in the areas of concern

Reflecting on the SynBio engineering mantra, also quoted by physicist Richard Feynman, "What I cannot create, I do not understand", the SCs understand that *creating is necessary but not sufficient* to understand the outcomes and products of SynBio. The gap between creating and understanding a SynBio organism is the driving force behind the question, "Do I understand what I can create?" (Schmidt, 2009). The SCs thus addressed the five SynBio areas and citizen science to shed light on gaps of knowledge currently present in SynBio for performing a reliable risk assessment for human health and the environment.

Genetic parts

Tools for predicting emergent properties of complex biological systems may not be sufficiently accurate or may not be available to risk assessors, which limits prediction and may impair the ability to accurately identify, test for or mitigate potential hazards. Additionally, existing modelling and simulation tools for complex biological systems may not quantify and assess the uncertainty of predictions (Breitling *et al.,* 2013) which contrasts with predictive tools used in other engineering areas and further development of corresponding tools for biological systems would be desirable.

Greater genetic distance between a SynBio organism and a comparator organism used in risk assessment results in decreased predictive abilities due to a higher number of novel interactions between modified and native parts.

The methods for submitting genetic modification data and genetic parts information to risk assessors remain non-standardised across EU Member States and internationally, and are largely in natural language. Such practices could limit the sophistication of quantitative analyses, data evaluation, efficiency and effectiveness of risk assessment. Ideally, such information should be submitted in computable form using a single application format for all Member States to facilitate transparency among all stakeholders, and to enable the application of the necessary prediction tools, including molecular and organismal systems biology methods for the modelling of complex biological systems (natural and engineered).

Minimal cells and designer chassis

Robustness, a well understood concept in engineering, is a relatively new concept in bioengineering. The concept of biological robustness is not yet fully clarified (Kitano, 2007). In traditional engineering disciplines, the robustness of a system is generally considered a positive feature, however, for biosafety, parts, devices and systems that extend robustness and environmental range of a chassis, e.g., tolerance of a wider range of biotic and abiotic conditions, may be a negative feature because it may be a safety issue (Schmidt, 2009). In contrast, fragility (i.e., lack of robustness) of a biological system potentially reduces its predictability and might impair risk assessment. The resulting trade-off is difficult to operationalise in a general framework.

In contrast to traditional engineered systems, the fundamental properties of engineered living systems can change over time, as a result of evolution and changes at the genetic level. The likelihood of unexpected evolution and unpredictable behaviour of an engineered microbe (an empty chassis would not be released, but rather complete systems, i.e. a chassis plus payload) if released into the environment is reduced if it is less fit (although it is not yet clear how fitness and robustness should be distinguished in this context, because they are related, but not identical). The extent of reduction in evolutionary potential as a consequence of reduced fitness is speculative and different for each organism and genetic payload. For engineering purposes, the ideal, but unattainable, threshold is zero evolution, i.e. no change in a chassis organism’s genome over time. It is currently not known how close to neutral or even zero evolution can be achieved. The challenge of defining biological robustness is difficult, because zero evolution corresponds to maximal genetic robustness, but may be most efficiently implemented by aiming for zero fitness, i.e. maximal fragility upon environmental release; fragility, in turn, should be implemented in a robust way (e.g., as a reliable safety lock), to avoid evolutionary escape. Moreover, evolution is an inherent feature of living systems, determined by the fundamental property of error-prone self-replication, even though evolutionary rates may potentially be reduced in engineered systems (Zakeri and Carr, 2015).

"In the end, safety is decided by humans" (Fischhoff *et al.,* 1978) and an acceptable level of risk must be assessed based on agreed thresholds using data generated from agreed protocols and metrics, and interpreted in the context of socioeconomic considerations and value judgements. There are many suggestions for metrics, but little agreement, and consequently, metrics are currently lacking for use in decision-making. For example, Mandell *et al.* (2015) consider that mutational escape frequency under laboratory growth conditions is a necessary but insufficient metric to evaluate biocontainment strategies.

Protocells

Protocells do not exhibit the full set of characteristics needed for passing the definition threshold of living organisms. They are more or less considered as chemistry systems and fall under the risk assessment and regulation of chemicals.

Knowledge gaps can be envisaged in the following three cases:

- These protocells could, for a limited time, interact synergistically with real living organisms (Lentini *et al*., 2014). To date, there is little or no information about the behaviour, impact and evolutionary ramifications of such systems consisting of organisms and chemical non-living systems.
- Protocells could, in the not so distant future, be further engineered to fully pass the definition threshold of living organisms. In this case, a form of life that is not directly related to any other pre-existing organisms would be generated, which means that no information would be available to evaluate the interaction between newly created and naturally evolved life forms. Autonomous, replicating chemical systems, which react dynamically to changes in their environment: hazardous properties of these cells should be assessed in the context of their intended use (contained use activity versus applications involving intentional release into the environment). Additionally, allergenicity, pathogenicity, biological stability, etc. must also be considered (Bedau *et al.*, 2009). The framework for risk assessment of these cells should begin with, but not necessarily be confined to, the methodology used for risk assessment of both GMO and non-GMO biological organisms.
- It is known that certain bacteria produce, under some circumstances, small lipid vesicles (Tetz *et al.,* 1993) and load them with peptides (Schrempf *et al.,* 2011) and chunks of RNA and DNA (Biller *et al.,* 2014), resulting in vesicles similar to protocells. Currently, it is not fully understood why this occurs in bacteria and to what extent it is an evolutionary advantage. Protocells, once released in the environment, could inadvertently mimic these natural vesicles and interfere in yet unknown biological functions. Biller *et al.,* 2014 noted: "The ability of vesicles to deliver diverse compounds in discrete packages adds another layer of complexity to the flow of information, energy, and biomolecules in marine microbial communities."

Xenobiology

The effects of non-standard biochemical molecules/systems, e.g., XNA, alternative base pairs, etc., in living cells should be evaluated to ensure safe deployment for applications in human health, agriculture and the environment.

The potential toxicity and allergenicity of novel xenobiological compounds should be evaluated (Schmidt and Pei, 2011).

Organisms engineered with xenobiology could, e.g., exhibit changes in evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases and predation. As with any GMO, such changes should be quantitatively and qualitatively characterised to support risk assessment. These data should be made available to the institutional biosafety boards and national biosafety authorities.

Xenobiology may be used to enhance biosafety engineering (semantic biocontainment), e.g., through the application of biological orthogonal systems, such as genetic firewalls. These novel biocontainment systems are, in general, expected to be more reliable if more xenobiological changes are introduced, hence the phrase "the farther the safer" (Marlière, 2009). For example, a full re-shuffling of the triplets of the genetic code, in combination with a new set of non-canonical proteinogenic amino acids, novel base pair combinations and a different backbone, is considered "farther away" from the original organism than if only one of these changes had been introduced. Each time another change is done, the probability, e.g., for horizontal gene flow, is further reduced. One important gap, however, is the lack of a *metric* to measure the structural (evolutionary) distance between the original (natural) and the engineered xenobiological organism. Establishing such a metric will be of paramount importance to the development, design, testing and deployment of novel biocontainment systems based on xenobiology.

Currently, the only metric is the evaluation of the escape frequency of engineered organisms (e.g., for auxotrophies). In an important study testing the biocontainment of GMOs by synthetic protein design, Mandell *et al.* (2015) stated*: "Our results demonstrate that mutational escape frequency under laboratory growth conditions is a necessary but insufficient metric to evaluate biocontainment strategies."* This metric has at least two major shortcomings, first: the detection limit to assess the escape frequency is about 10^{-11}. The detection limit however, should be several orders of magnitude below this value to generate useful information for deciding on the validity of a proper containment system. The second shortcoming is the lack of standardised media to test the escape frequencies for several potential escape environments. In a recent paper on the experimental evaluation of a genetic firewall, Rovner *et al.* (2015) tested their engineered strains on blood agar and soil extracts to allow for an improved evaluation of the validity of the firewall. These environmentally aware additional tests should be extended and standardised to allow for better predictability and comparability.

While escape frequency tests the survival and growth of (auxotrophic) strains, another test battery should be set up to assess the probability of horizontal gene flow from the novel strain to natural organisms, establishing the similar metrics, rigour and standards including escape frequencies.

DNA synthesis and genome editing

The new technologies for DNA synthesis and genome editing underlie many of the applications of SynBio discussed above; most importantly, those covered in the sections of genetic parts and minimal cells, which both depend on the progress of DNA synthesis and genome editing. The use of genome editing methods in a multiplexed fashion allow the simultaneous generation of large number of variants, the genome-wide modification of organisms and a more accurate and precise change to the genomes of living organisms than those obtained by traditional, targeted genetic modification techniques according to current regulations. This considerably hampers the case-by-case approach by which living organisms obtained by traditional targeted genetic modification techniques were risk assessed. The scale and speed at which new and complex organisms will be generated might create additional challenges from a risk assessment standpoint, because a case-by-case risk assessment, as currently adopted for living organisms obtained by traditional genetic modification techniques, may no longer be feasible.

Citizen science

In principle, any amateur or citizen biologist (DIY biologist) in Europe who plans to carry out work with SynBio or GMOs in Europe has to undergo the same safety regulations as researchers in traditional institutions. Thus, the same caution and safety rules apply to citizen scientists. A gap in knowledge and awareness of established biosafety rules in the various European countries may arise and thus, reduce compliance. According to two recent surveys in the USA (Grushkin *et al.*, 2013) and Europe (Seyfried *et al., 2*014), the do-it-yourself (DIY) biology groups comply with national laws and guidelines and actively try to increase awareness for biosafety and ethical issues within their community. The Grushkin *et al.* 2013 report, however, represented only those who voluntarily participated via self-selection on an online survey. In the Seyfried *et al.* 2014 study, the authors interviewed and visited labs in several European cities and identified groups that actively promoted themselves over the web, and participated actively in European community meetings. While both reports provide reassurance that biohackers are constructive and aware of the dangers of biotechnology, they do not address individuals or groups working outside of these groups. There is a potential risk that, without appropriate oversight, activities of a rogue biohacker may lead to biosecurity and/or biosafety issues.

3.4.Introduction Research recommendations on the main scientific gaps

3.4.1. Research recommendations related to gaps in six novel SynBio developments

Genetic parts

- Support research
    - To characterise the novel interactions between modified and native parts
    - To develop computational tools to predict emergent properties of SynBio organisms and their potential failure modes, including biological prediction tools that explicitly incorporate the uncertainty of molecular and genetic information
    - Broad dissemination and training in such tools and knowledge resources.

- Research approaches to streamline and standardise across EU Member States the methods for submitting genetic modification data and genetic parts information, including systems biology models, to risk assessors. The level of detail of data to be provided should take into account the intended use (contained use versus deliberate release into the environment). Ideally, such information should be submitted in computable forms to facilitate transparency for all stakeholders, and to enable the application of the aforementioned prediction tools, including systems biology models.
- Develop brief guidelines for risk assessors on the evaluation of potential emergent properties of genetically engineered systems.
- Research the use of GMOs with a proven safety record as acceptable comparators for risk assessment so that the baseline state of safe organisms can advance in step with the complexity of new modifications. Reliance solely on non-GMO organisms, as opposed to GM organisms with a history of safe use, would prevent the advance of baseline risk assessment controls. Alternatively, the use of GM organisms with a record of safety may better reflect the current understanding of risks.

Minimal cells and designer chassis

Additional level of safeguards may be 'biosafety-aided design' to investigate the biosafety of modules at the design stage. Software designers in the SynBio community are currently developing safeguards to help scientists prevent unintentional creation of unsafe organisms before the system is actually built, but this is restricted to the level of individual sequences, such as the detection of matches to virulence factors (Moe-Behrens *et al.*, 2013). There is a need for the development of tools for reliable prediction of emergent safety issues at the systems level.

Current strategies are insufficient (Mandell *et al.*, 2015) as they:

- impose evolutionary pressure on the organism to 'evolve out' the safeguard by spontaneous mutagenesis or horizontal gene transfer, or:
- can be circumvented by environmental supplementation using compounds scavenged from the receiving environment.

The current consensus is that the bare minimum for safety of a deployed genetically modified microorganism (GMM) for intentional environmental release (commercial, experimental or environmental purposes) should consist of multiple safety devices of different types (Presidential Commission for the Study of Bioethical Issues, 2010). The SCs suggest the establishment of a public repository of well-characterised engineered safe chassis and safety devices (e.g., toxin-antitoxin systems, altered genetic codes) that ideally can be combined, in a modular manner, to allow for multi-layer safety systems that are implemented for specific requirements. Relevant stakeholders should agree upon a clear concept as to how this repository is organised and managed.

Juhas *et al.* in 2012 suggested that the next big challenge in SynBio is developing clever systems for robust growth and radical genome changes that aim at producing useful products. Changing the translational genetic code, including codons of essential genes, could lead to generations of cells resistant to currently existing viruses or incapable of survival outside the laboratory environment. While adding modules might make the chassis less fit, increasing bioreactor robustness might also increase environmental robustness. Additional research is required to establish the best approach to deal with this trade-off.

Standardised techniques should be used to generate comparative data across both organisms and environments for use in quantitative biocontainment assessment. An example is a conjugation escape assay (Mandell *et al.*, 2015) to assess how DNA transfer within an ecosystem enables a GMO to escape biocontainment. The establishment of further standardised techniques and protocols would be useful.

Further work is required on designing synthetic constructs and microbes that are intentionally out-competed over time. For this research to progress, more quantitative data are needed on how GMMs perform in sample environments (Wright *et al.*, 2013). The current lack of in-depth testing makes it difficult to accurately assess which safety mechanisms and designs are best at preventing ecological invasion and horizontal gene transfer.

Chassis organisms and their genetic payloads should be engineered for reduced rates of evolution (increased robustness), while at the same time ensuring their fragility upon accidental release (decreased robustness). Further fundamental research on quantifying and qualifying the evolutionary change of phenotypes through time is required to understand and predict how these two demands can be simultaneously satisfied. Zakeri & Carr (2015) recently presented a conceptual analysis of evolution as a "significant and absolute barrier" for SynBio, with a focus on the decline in functionality of engineered systems as a result of evolution. Additional work (both theoretical and experimental) is needed to determine how these ideas apply to more complex real-world scenarios, with multiple and sometimes mutually exclusive objectives and functionalities.

Protocells

The recommendations address the three identified gaps in protocell interaction.

- More information is necessary to assess the implications, and the environmental and evolutionary consequences of a collaborative interaction between non-living protocells and living organisms as described in Lentini *et al.,* 2014. Protocells are possible functionality enhancers for living cells, delivering "prosthetic" capabilities not present in the collaborating cells. For example, the host range should be identified to avoid unlikely, but not impossible, infections by protocells, especially if they differ from natural cells (Schmidt *et al.*, 2009). Importantly, it is necessary to determine the specificity of symbiotic interactions between protocells and natural cells and to determine the outcome of unforeseen interactions of other cells with protocells.
- Preparation for the possibility of engineered protocells that are life-like entities, i.e. moving from protocells to real cells. This may prove difficult for risk assessors to judge the risk of new life forms on human health and the environment, e.g., allergenicity, pathogenicity, biological stability, etc., because there are no natural counterparts and all information should be newly generated.
- More research is necessary on the ecological and evolutionary role of natural vesicles containing peptides, RNA and DNA. Because these natural vesicles are supposed to play a role in bacterial defence, protocells could inadvertently trigger, or interfere with, natural inter-bacterial communication pathways with an unclear outcome.

Besides, it is unclear to what extent existing regulations, such as the GMO regulations, or the guidelines on invasive species will be used or whether it will be necessary to create entirely new regulations and risk assessment guidelines.

Xenobiology

Based on the aforementioned scientific gaps, the SCs recommend the following research priorities:

- Investigation of the potential toxicity and allergenicity of novel xenobiological compounds (i.e., the various non-canonical nucleic acids, amino acids and related molecules).
- Even when each individual chemical class of xeno-compounds (e.g., HNA, GNA) is initially characterised and comprehensively tested (e.g., for toxicity and allergenicity), a risk assessment is needed for emergent properties. In the future, for proven safety records of particular classes of xeno-compounds, applications of such classes are tested in the same way as classical DNA modifications, namely, based on a case-by-case assessment of the modified genetic information only.
- Establish a methodology to quantitatively and qualitatively characterise xenobiology organisms with respect to evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses and diseases or predation.
- To enable and enhance biosafety engineering (e.g., with genetic firewalls):
    - Development of clear and reliable metrics to measure the escape frequency of different types of semantic containment (e.g., the use of different genetic codes, or alternative biochemistries of key informational biopolymers such as nucleic acids or amino acids).
    - Improvement of the mechanistic understanding of underlying principles of semantic containment, to allow for a reliable prediction of the strength of semantic containment strategies.
    - Improvement and standardisation of testing platforms for existing metrics for assessing the escape frequency well beyond rates of 10^{-11} escapes per colony forming unit currently measurable in laboratory conditions and potential (unintended) target environments (e.g., soil, blood, water, etc.).
    - Improvement and standardisation of existing metrics to measure the horizontal gene flow from novel strain to natural organisms establishing similar metrics, rigour and standards as in the case of escape frequencies.
    - Further development of xenobiology-based biocontainment systems such as genetic firewalls using the metrics and standardised testing platforms mentioned above.

DNA synthesis and genome editing

The reader is referred to the recommendations under ‘genetic parts’ and ‘minimal cells and designer chassis’.

The increasing use of gene drive technology, though outside the scope of this Opinion (section 3.1.4), would require an in-depth analysis, including a detailed assessment of its implications for risk assessment methodology and its potential impact on biodiversity and the environment.

Citizen science

The SCs recommend the development of strategies on how to further increase the awareness and compliance of citizen scientists with national biosafety rules and codes of ethics. Existing tools, like the “ask a biosafety officer” approach should be further promoted and possible new ones added. A potential beneficial path would be to allow for an environment where citizen scientists have more opportunities to collaborate on a case-by-case basis with traditional institutions, either virtual or physical. Further support, especially for newcomers, to get for example an introductory course into laboratory biosafety, could also be considered.

4. OPINION

This Opinion is the third in a series of three on Synthetic Biology (SynBio) responding to questions from the European Commission. The overall, legal and scientific background underlying these questions from the Commission were discussed in the first Opinion and a definition of SynBio was proposed. In the second Opinion, the Scientific Committees (SCs) addressed the five subsequent questions focusing on the implications of likely developments in SynBio on human and animal health and the environment and on determining whether existing health and environmental risk assessment practices of the European Union for Genetically Modified Organisms (GMOs) are also adequate for SynBio. Additionally, the SCs were asked to provide suggestions for revised risk assessment methods and risk mitigation procedures, including safety locks.

The SCs confined the scope of its analysis to the foreseeable future (up to 10 years, *i.e.* until 2025), acknowledging that its findings should be reviewed and updated after several years, depending on the progress of SynBio technology. Outside the scope of the current mandates are specific, thorough analyses of social, governance, ethical and security implications of SynBio as well as human embryonic research.

Recognising that SynBio evolved from and shares much of the methodologies and tools of genetic engineering, it is considered in this Opinion, as well as in the previous ones, that the risk assessment methodology of contained use activities and activities involving the deliberate release of GMOs are built on principles outlined in the Directives 2001/18/EC and 2009/41/EC and in Guidance notes published in Commission Decision 2000/608/EC.

The SCs focus their analysis on five research areas and one trend in SynBio: genetic part libraries and methods, protocells, minimal cells and designer chassis, xenobiology, DNA synthesis and genome editing and citizen science.

Opinion III is focused on answering the following questions on SynBio:

9. The SCENIHR, SCHER, SCCS are asked to review the state of the scientific knowledge concerning specific risks to the environment and synthesise it following the procedure and the requirements mentioned in the Decision XI/11 of the Convention of Biodiversity and include the synthesis in its Opinion.

Impacts on biological diversity and conservation

The SCs analysed how key areas of application of SynBio may affect, either in a positive or in a negative way, the objectives of the CBD. They further analysed impacts on the Aichi Biodiversity Targets for the 2011-2020 period. The following synthesis concentrates on potential negative impacts on biodiversity and conservation:

- The increased demand for specific feedstock might have negative impacts on biodiversity and conservation, e.g., through increased extraction of biomass from agricultural land resulting in decreased soil fertility or through extraction of biomass from the natural environment. This may affect Aichi Targets 4 and 15.
- Various applications may lead to accidental release of SynBio organisms into the environment and negatively affect biodiversity and conservation.
- The ability of designing and producing improved plant varieties based on genome sequence data could reduce the focus on conserving old land races and the need to

preserve wild relatives, once they are sequenced. Artificial diversity could lead to lack of perceived value of natural biodiversity. This is considered to affect Aichi Targets 1 and 13. Likewise, de-extinction research may have a destabilising effect on conservation, potentially resulting in species loss, due to potentially reduced focus on species and habitat preservation and underlying causes for biodiversity loss support for in situ conservation and existing protected areas might be reduced. This may affect Aichi Targets 1 and 13.

- SynBio alternatives for chemical products and industrial processes might not actually be more sustainable than traditional products.

Specific risks to the environment

Risks to the environment were analysed on the basis of Opinion II, key EU Framework projects and pertinent literature. Generic risk factors identified were mostly discussed above in relation to impacts on biodiversity and conservation. These risk factors are related to accidental release, persistence of SynBio organisms designed or environmental release, such organisms becoming invasive or disrupt food webs, transfer of genetic material from vertical gene flow or horizontal gene transfer and potential impacts on biodiversity and ecosystems from ”de-extinction”. In general, these risks need a more thorough analysis of the probability at which they may occur and the impacts they may have.

Similar to Opinions I and II, the analysis of specific risks to the environment was done for each of six novel SynBio developments: 1) Genetic part libraries and methods; 2) Minimal cells and designer chassis; 3) Protocells and artificial cells; 4) Xenobiology: 5) DNA synthesis and genome editing; and 6) Citizen science. Table 2 shows the pertinent conclusions.

Table 2: Specific risks to the environment

SynBio development	Specific risk
Genetic parts	Increased frequency of use of uncharacterised components and/or the diversity of biological functions. Interactions of the parts may lead to emergent functions, presenting new challenges in predicting or testing for risks and in the identification of appropriate comparator organisms.
Minimal cells and designer chassis	Risk of endosymbiontic relationship with another organism and eventually the establishment of an organelle. Evolution of large virus from minimal cells.
Protocells	In the future, exposure to autonomous artificial cells surviving in the laboratory and in the environment might be possible. Although protocells are not alive, they can be engineered to intimately interact with living cells and enhance overall system functionality. Thus, novel biological functions may be designed without altering the DNA of these target organisms. The genetic information that controls internal functioning might mutate or be horizontally transferred. Thus, a population of protocells with different genetic information could undergo selection and new protocells may arise.
Xenobiology	New variants based on non-standard biochemical systems may present unknown risks. The degree of risk reduction through the genetic firewall requires characterisation.
DNA synthesis and genome editing	The increased speed of modifications through these technologies might pose challenges to risk assessment, while not in itself

	creating new risks.
Citizen science	The probability of unintentional harm might increase, because more people are starting to actively work with biological material outside of conventional laboratory and institutional settings.

Prevention of risks

Risks from SynBio organisms may be prevented wholly or in part by a) Design of less competitive organisms by changing metabolic pathways; b) Replacing metabolic pathways with others that have an in-built dependency on external biochemicals; c) Design of evolutionary robust biological circuits; d) Use of biological systems based on an alternative biochemical structure to avoid e.g., gene flow to and from wild species; e) Design of protocells that lack key features of living entities, such as growth or replication. Currently available safety locks used in genetic engineering such as genetic safeguards (e.g., auxotrophy and kill switches) are not yet sufficiently reliable for SynBio. Genetic firewalls may improve containment compared with classical genetic engineering. However, no single technology reliably manages all biosafety risks and new approaches and combinations of existing and upcoming new strategies will be necessary including new forms of biocontainment and additional layers of containment using orthogonal systems.

Mitigation of risks

Mitigation is defined by the SCs as risk reduction measures taken after deliberate or accidental release of SynBio organisms, components or products and when all biocontainment processes, safety locks and other preventive measures have failed. Organisms, resulting from SynBio techniques or not, may not be retrieved once released. Given the difficulties in preventing a biological incident of any type, the main goal of contingency management should be to avoid and/or mitigate an event. In specific and high-risk cases, a prepared, efficient and proportional international response may limit the size and scope of such releases as well as the implementation of WHO IHR standards, including the prior assessment of the necessity for international notification.

10. What are the major gaps in knowledge to be filled for performing a reliable risk assessment in the areas of concern?

The SCs addressed five SynBio research areas and citizen science to shed light on gaps of knowledge necessary to perform a reliable risk assessment for human health and the environment currently present in SynBio. Table 3 shows the conclusions.

Table 3: Gaps in knowledge

SynBio development	Gap
Genetic parts	• Tools for predicting emergent properties of complex biological systems may not be sufficiently accurate or may not be available to risk assessors • The methods for submitting genetic modification data and genetic parts information to risk assessors is yet unstandardised across EU member states and internationally and are largely natural language submissions. Such practices could limit the sophistication of quantitative analyses, data evaluation, efficiency and effectiveness of risk assessment.

Minimal cells and designer chassis	How to define and engineer biological robustness with the aim to move closer to neutral or even zero evolution.
Protocells	• There is little to no information about the behaviour, impact and evolutionary ramifications of interactions of systems consisting of organisms and chemical non-living systems. • Unknown hazardous properties of future autonomous, replicating chemical systems, including, allergenicity, pathogenicity, biological stability. • Lack of knowledge on behaviour of "natural protocells" i.e. lipid vesicles produced by bacteria and loaded with peptides, RNA, DNA, which may be a comparator to synthetic protocells.
Xenobiology	• Unknown effects of non-standard biochemical molecules/systems, e.g., XNA, alternative base pairs, etc., in living cells. • Unknown potential toxicity and allergenicity of novel xenobiological compounds. • Lack of data supporting risk assessment such as change in evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases or predation. • Lack of a clear and reliable *metric* to measure the escape frequency of different types of semantic containment (e.g., the use of different genetic codes, or alternative biochemistries of key informational biopolymers such as nucleic acids or amino acids). • Insufficient mechanistic understanding of underlying principles of semantic containment, to allow for a reliable prediction of the strength of semantic containment strategies is missing.
DNA synthesis and genome editing	The increased speed of modifications might pose challenges to risk assessment mainly because administrative procedures might not be able to cope with a large number of rapidly created engineered organisms.
Citizen science	Knowledge gap whether citizen scientists reliably comply with the established biosafety rules.

11. SCENIHR, SCHER, and SCCS are requested to provide research recommendations on the main scientific gaps identified The recommendations should also include methodological guidance on the experimental design and on the requirements of the proposals, in order to ensure data quality and comparability, as well as the usability of the results for risk assessment.

The SCs previously recommended risk assessment related research in Opinion II:

- Support research that
  - Characterises the function of biological parts
  - Develops computational tools to predict emergent properties of SynBio organisms and their potential failure modes
  - Broadly disseminates knowledge and trains scientists.
- Streamline and standardise the methods for submitting genetic modification data and genetic parts information across EU member states to risk assessors, which should be transparent and available to all stakeholders.
- Encourage the use of GMOs with proven safety records as acceptable comparators for risk assessment, i.e. the baseline state of safe organisms can advance with the complexity of new modifications. Reliance solely on non-GMO organisms, as opposed to GMOs with a history of safe use would prevent the advance of baseline risk

assessment controls. In contrast, use of GMOs with a record of safety may better reflect the current understanding of risks.

- Support additional research and debate towards the development of sufficiently sophisticated risk assessment tools to match the advances in technology assessed, to avoid an imbalance between risk assessment and technology that might negatively impact economic and health benefits of the technology and jeopardise the quality of safety protections.
- Support a Biosafety clearinghouse on bioparts, devices and systems to support risk assessment of genetic circuits generated with biological parts, devices and systems.
- The SCs suggest sharing relevant information about specific parts, devices and systems with risk assessment practitioners.

The following research recommendations for the improvement of risk assessment follow from the gaps identified for each of the six novel SynBio developments:

General recommendations

Research on standardised techniques to monitor biocontainment and survival in environments outside the bioreactor and to generate comparative data for use in quantitative biocontainment assessment. Additional research is required to establish the best ways of dealing with the trade-off that, whilst adding biosafety modules might make the chassis less fit, increasing fitness in the bioreactor might also increase environmental fitness. Further work is also required on how to design synthetic constructs and microbes that will be intentionally out-competed over time. For this research to progress, more quantitative data are needed for how GMOs perform in sample environments.

Genetic parts

- Support research
    - To characterise the interactions between modified and native parts
    - To develop computational tools to predict emergent properties of SynBio organisms and their potential failure modes, including biological prediction tools that explicitly incorporate the uncertainty of molecular and genetic information
    - Broad dissemination and training in such tools and knowledge resources
- Research approaches to streamline and standardise the methods for submitting genetic modification data and genetic parts information, including systems biology models, to risk assessors across EU member states. Ideally, such information should be submitted in computable form to facilitate transparency with all stakeholders involved in the risk assessment process, and to enable the application of the aforementioned prediction tools, including systems biology models.
- Develop brief guidelines for risk assessors on how to evaluate potential emergent properties of genetically engineered systems.
- Research the use of GMOs with a proven safety record as acceptable comparators for risk assessment such that the baseline state of safe organisms can advance in step with the complexity of new modifications. Reliance solely on non-GMO organisms, as opposed to GM organisms with a history of safe use, would prevent the advance of baseline risk assessment controls. On the other hand, use of GM organisms with a record of safety may better reflect the current understanding of risks.

Minimal cells and designer chassis

- Research the introduction of biosafety of modules at the design stage. There is a need to develop tools for reliable prediction of emergent safety issues at the systems level. The natural extension of this is the design and testing of biological chassis for safety and sustainability, with attention to limiting chassis survivability and genetic exchange on release.
- There is a need to engineer chassis organisms and their genetic payloads for reduced rates of evolution (increased genetic robustness), while at the same time ensuring their fragility upon accidental release (decreased environmental robustness). Further fundamental research on quantifying and qualifying the evolutionary change of phenotypes through time is required to understand and predict how these two demands can be satisfied at the same time.

Protocells

- More information is needed to assess the implications, as well as the environmental and evolutionary consequences of a collaborative interaction between non-living protocells and living organisms, including the host range and the specificity of collaborative interactions between protocells and natural cells.
- If protocells become life-like entities, methods should be developed to assess their risk e.g., allergenicity, pathogenicity, biological stability, etc. in the absence of biological counterparts. Regulatory consequences should be investigated as well.
- More research is necessary to learn more about the ecological and evolutionary role of natural vesicles containing peptides, RNA and DNA.

Xenobiology

- Even when each individual chemical class of xeno-compounds (e.g., HNA, GNA) initially is characterised and tested comprehensively (e.g., for toxicity and allergenicity), a risk assessment is needed for emergent properties. In the future, in case of a proven safety record of particular classes of xeno-compounds, applications of such classes should be tested similarly to classical DNA modifications, namely based on a case-by-case assessment of the modified genetic information only.
- Establish a methodology to quantitatively and qualitatively characterise xenobiology organisms with respect to evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to viruses, diseases or predation.
- Develop a clear and reliable *metric* to measure the escape frequency of different types of semantic containment. Improve and standardise testing platforms for existing metrics for assessing the escape frequency well beyond rates of 10^{-11},based on typical cell densities and fermenter sizes, in laboratory conditions and potential (unintended) target environments (soil, blood, water, etc.).
- Improve the mechanistic understanding of underlying principles of semantic containment, to allow for a reliable prediction of the strength of semantic containment strategies. Further develop xenobiology-based biocontainment systems such as genetic firewalls using the metrics and standardised testing platforms mentioned above.

DNA synthesis and genome editing

The reader is referred to the recommendations under 'genetic parts' and 'minimal cells and designer chassis'.

Citizen science

The SCs recommend the development of strategies on how to increase the awareness and compliance of citizen scientists with national biosafety rules and codes of ethics including collaboration with acknowledged institutions and training. Existing tools, like the "ask a biosafety officer" approach should be further promoted and possible new ones added.

Additional research recommendations

Additional research recommendations for the improvement of risk assessment can be identified from the section on impacts on biodiversity and conservation and specific risks to the environment:

- Research on impacts from accidental or intentional introduction of SynBio organisms into the environment with emphasis on:
  - Effects on habitats, food webs and biodiversity,
  - The difference in physiology of natural and synthetic organisms,
  - Vertical or horizontal gene flow,
  - Survival, persistence, ecological fitness and rate of evolutionary change.
- Research on the impacts from "de-extinction" and the debate around it.
- Research on the containment strategies to prevent unintentional release of or exposure to organisms resulting from SynBio techniques. The SCs recommend exploring a clear strategy for the analysis, development, testing and prototyping of applications based on new forms of biocontainment and additional layers of containment using orthogonal systems. Barriers can be physical, biological or semantic.
- The environmental performance of SynBio processes and products should remain a research focus considering the full product life cycle. The development of a flexible assessment methodology is needed in which criteria for human and environmental health, safety and sustainability can be selected.
- An emerging technology that uses similar techniques to the ones that are commonly applied in genome editing for SynBio applications are the so-called "gene drives". However, for the purposes of this Opinion, gene drives are not considered as falling under the definition of SynBio. While the methods used are related, gene drives aim at modifying the genetic composition of populations, not of individual organisms; an analysis of the risks and implications of "gene drives" is therefore outside the scope of this Opinion. Nevertheless, the increasing use of gene drive technology would certainly require a similar in-depth analysis, including a detailed assessment of its implications for risk assessment methodology and its potential impact on biodiversity and the environment.

Prioritisation of impact assessments can be based on prior knowledge available.

5. MINORITY OPINION

None.

6. CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this Opinion was opened on the website of the non-food scientific committees between 16 July 2015 and 16 September 2015.

12 organisations and individuals (contributing 61 comments in total) participated in the public consultation providing input to different chapters and subchapters of the Opinion. Among the organisations participating in the consultation were universities, institutes of public health, NGOs and public authorities.

Each contribution was carefully considered by the Scientific Committees and the scientific Opinion has been revised to take account of relevant comments.

The text of the comments received and the response provided by the Scientific Committees is available here:

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_28_en.htm

7. ABBREVIATIONS AND GLOSSARY OF TERMS

- Biosafety level (BSL)
- Convention of Biodiversity (CBD)
- Cartagena Protocol on Biodiversity (CPB)
- Clustered Regularly Interspaced Short Repeats (CRISPR)
- Decision XI/11 of the Convention of Biodiversity (COP Decision XI/11)
- De-extinction (Bringing extinct species back to life)
- European Centre for Disease prevention and Control (ECDC)
- European Chemicals Agency (ECHA)
- European Commission (EC)
- European Food Safety Authority (EFSA)
- European Medicines Agency (EMA)
- European Union (EU)
- Genetically modified microorganisms (GMM)
- Genetically modified organisms (GMOs)
- Horizontal gene transfer (HGT, transfer of genes between organisms independent of sexual or asexual reproduction)
- International Genetically Engineered Machine (iGEM)
- International Health Regulations (IHR)
- Living Modified Organisms (LMOs)
- Multiplex Automated Genome Engineering (MAGE)
- Ministry of Science and Technology (MOST)
- Nagoya Protocol (NP)
- National Institutes of health (NIH)
- Natural Language (human language, in contrast to computer language)
- New plant breeding techniques (NPBTs)
- Organisation for Economic Co-operation and Development (OECD)
- Scientific Committee (SC)
- Scientific Committee on Consumer Safety (SCCS)
- Scientific Committee on Health and Environmental Risks (SCHER)
- Semantic containment (Use of biocontainment systems through the implementation of genetic language which is not compatible with natural biological systems)
- Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA)
- Synthetic Biology (SynBio)
- Transcription activator-like effector nucleases (TALENs)
- United Nations Convention on Biological Diversity (CBD)
- Vertical gene transfer (Transmission of genes from the parental generation to offspring via sexual or asexual reproduction)
- Xeno Nucleic Acids (XNA)
- World Health Organisation (WHO)

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9. ANNEXES

9.1. Annex I Questions from the mandate

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) in association with Scientific Committee on Consumer Safety (SCCS), Scientific Committee on Health and Environmental Risks (SCHER), request for a joint scientific opinion on SynBio.

Scope and definition of the phrase "SynBio"

1. What is SynBio and what is its relationship to the genetic modification of organisms?
2. Based on current knowledge about scientific, technical, and commercial developments, what are the essential requirements of a science-based, operational definition of "SynBio"? These requirements should comprise specific inclusion and exclusion criteria, with special attention given to quantifiable and currently measurable ones.
3. Based on a survey of existing definitions, to which extent would the definitions available meet the requirements identified by the Committee as fundamental and operational?

Methodological and safety aspects

4. What are the implications for human and non-human animal health and the environment of likely developments in SynBio resulting or not in a genetically modified organism as defined in the Directive 2001/18/EC?
5. Are existing methodologies appropriate for assessing the potential risks associated with different kinds of activities, tools, products and applications arising from SynBio research?
6. If existing methodologies are not appropriate to assess the potential risks associated with activities related to and products arising from SynBio research, how should existing methodologies be adapted and/or completed?
7. How, when, and to what extent can safety (safety locks) be inherently built into products of SynBio?
8. The SCENIHR, SCHER, SCCS are asked to draw the blue print of a general procedure/strategy for designing inherently safe applications of SynBio.

Research priorities

9. The SCENIHR, SCHER, SCCS are asked to review the state of the scientific knowledge concerning specific risks to the environment and synthesise it following the procedure and the requirements mentioned in the COP Decision XI/11 of the Convention of Biodiversity and include the synthesis in its opinion.
10. What are the major gaps in knowledge which are necessary for performing a reliable risk assessment in the areas of concern?
11. SCENIHR, SCHER, and SCCS are requested to provide research recommendations on the main scientific gaps identified The recommendations should also include methodological guidance on the experimental design and on the requirements of the proposals, to ensure data quality and comparability, as well as the usability of the results for risk assessment.

9.2. Annex II Abstract of Opinion I

This Opinion is the first of a set of three Opinions addressing a mandate on Synthetic Biology (SynBio) from DG SANCO, DG RTD, DG Enterprise and DG Environment requested to the three Scientific Committees (SCs). This first Opinion concentrates on the elements of an operational definition for SynBio. The two Opinions that follow will focus on risk assessment methodology, safety aspects and research priorities, respectively. This first opinion lays the foundation for the two other opinions with an overview of the main scientific developments, concepts, tools and research areas in SynBio. Additionally, a summary of relevant regulatory aspects in the European Union, in other countries such as the USA, Canada, South America, China, and at the United Nations is included. Although security issues concerning SynBio are important, the terms of reference pertain exclusively to safety and, thus, security issues will not be addressed in any of the three Opinions.

In brief, the answers to the first three questions asked in the mandate are:

1. What is Synthetic Biology and what is its relationship to the genetic modification of organisms?

Over the past decade, new technologies, methods and principles have emerged that allow for faster and easier design and manufacturing of GMOs, which are referred to as Synthetic Biology (SynBio). SynBio is currently encompassed within genetic modification as defined in the European Directives 2001/18/EC and 2009/41/EC and will likely remain so in the foreseeable future.

Current definitions of SynBio generally emphasise modularisation and engineering concepts as the main drivers for faster and easier GMO design, manufacture and exploitation. However, the operational definition offered here addresses the need for a definition that enables risk assessment and is sufficiently broad to include new developments in the field. Therefore, for the purpose of these Opinions, this is the operational definition derived from a working understanding of SynBio as a collection of conceptual and technological advances:

SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms.

2. Based on current knowledge about scientific, technical, and commercial developments, what are the essential requirements of a science-based, operational definition of “Synthetic Biology”? These requirements should comprise specific inclusion and exclusion criteria, with special attention given to quantifiable and currently measurable ones.

The opinion proposes an ‘operational’ definition based on present knowledge and understanding of the field of SynBio. However, this definition may change as the understanding of the SynBio concepts, tools and applications evolves.

SynBio includes any activity that aims to modify the genetic material of living organisms as defined in the Cartagena Protocol on Biodiversity. This does not exclude the consideration of non-viable, non-reproducing goods and materials generated by or through the use of such living genetically modified organisms (GMOs). Genetic

Modification (GM) involves the modification of living organisms with heritable material that is independent of the chemical nature of the heritable material and the way in which this heritable material has been manufactured. SynBio uses all available technologies for genetic modification, but in particular, aims at a faster and easier process, which also increases predictability.

It is difficult to accurately define the relationship between genetic modification and SynBio on the basis of quantifiable and currently measurable inclusion and exclusion criteria. Thus, in addition to the definition, a list of specific criteria was considered reflecting that SynBio covers any organism, system, material, product, or application resulting from introduction, assembly, or alteration of the genetic material in a living organism. Although these criteria are helpful guiding principles that specify whether or not a certain process, tool or product belongs to SynBio, none are quantifiable or measurable. Additional criteria including the complexity of the genetic modification, the speed by with modification was achieved, the number of independent modifications, or the degree of computational design methods used, alone nor in combination are also unable to unambiguously differentiate SynBio processes or products from GM.

3. Based on a survey of existing definitions, to which extent would the definitions available meet the requirements identified by the Committee as fundamental and operational?

A survey of 35 published definitions is provided in an annex to this Opinion. Existing definitions are focused on conceptual advances within the scientific community. However, these definitions are neither operational nor fundamental, because they are not based on quantifiable and currently measurable criteria. To address the deficiency in existing definitions and to enable our practical work on risk assessment, the science-based operational definition of SynBio above is suggested.

This definition has the advantage that it does not exclude the relevant and large body of risk assessment and safety guidelines developed over the past 40 years for GM work and extensions of that work, if needed, to account for recent technological advances in SynBio. Additionally, the present definition also allows for the rapidly advancing nature of GM technologies and important nuance that supports the need for on-going updates of risk assessment methods, which will be addressed in Opinion II.

9.3. Annex III Abstract of Opinion II

In Opinion I on synthetic biology (SynBio), the three non-food Committees of the European Union SCHER, SCENIHR, and SCCS answered the first 3 out of 11 questions from the European Commission on scope, definition and identification of the relationship between SynBio and genetic engineering, and the possibility of distinguishing the two.

In this second Opinion (Opinion II), the Scientific Committees (SCs) addressed the five subsequent questions focused on the implications of likely developments in SynBio on human and animal health and the environment and on determining whether existing health and environmental risk assessment practices of the European Union for Genetically Modified Organisms (GMOs) are also adequate for SynBio. Additionally, the SCs were asked to provide suggestions for revised risk assessment methods and risk mitigation procedures, including safety locks.

Because SynBio is a rapidly evolving technology, the SCs suggest that risk assessment of and methodology for SynBio must be revisited at regular intervals. Although it is outside the scope of the current mandate, some background considerations about the social, governance, ethical and security implications of SynBio are also provided.

SynBio shares several methodologies and tools with genetic engineering. In Opinion II, the SCs evaluated risk assessment methodology of use activities and activities involving the deliberate release of GMOs that are built on the principles outlined in Directives 2001/18/EC and 2009/41/EC and in the Guidance notes published in Commission Decision 2000/608/EC. These principles address the magnitude of potential hazards and adverse effects of genetic engineering on human health and the environment and on the probability that they might lead to hazards (exposure chain). Herein, the SCs assess six novel SynBio developments: 1) Genetic part libraries and methods; 2) Minimal cells and designer chassis; 3) Protocells and artificial cells; 4) Xenobiology: 5) DNA synthesis and genome editing; and 6) Citizen science (Do-It-Yourself biology (DIYbio)). Notably, complexity and uncertainty are characteristic parts of the risk assessment of SynBio and have lead the SCs to conclude that within the scope of current GMO regulations, risk assessment is challenging, e.g., because of the lack of ‘comparators’ and the increasing number of genetic modifications and engineered organisms.

This Opinion addresses questions 4-8 of 11 of the SynBio mandate:

Question 4: What are the implications for human and animal health and the environment of likely developments in SynBio resulting or not in a genetically modified organism as defined in the Directive 2001/18/EC?

New challenges in predicting risks are expected due to emergent properties of SynBio products and extensive genetically engineered systems, including, 1) the integration of protocells into/with living organisms, 2) future developments of autonomous protocells, 3) the use of non-standard biochemical systems in living cells, 4) the increased speed of modifications by the new technologies for DNA synthesis and genome editing and 5) the rapidly evolving DIYbio citizen science community, which may increase the probability of unintentional harm.

The framework for risk assessment of new SynBio developments may be addressed using current methodology used for GMO risk assessment. However, there are specific cases in which new approaches may be necessary. These include risks pertaining to 1) routes of exposure and adverse effects arising from the integration of protocells into living organisms and future developments of autonomous protocells, 2) new xenobiological variants and their risk on human health and the environment that should be engineered for improved biocontainment, 3) DNA synthesis and direct genome editing of zygotes which enables modifications in higher animals within a single generation, and 4) new multiplexed genetic modifications which increase the number of genetic modifications introduced in parallel by large-scale DNA synthesis and/or highly-parallel genome editing and will increase the genetic distance between the resulting organism and any natural or previously modified organism.

Question 5: Are existing methodologies appropriate for assessing the potential risks associated with different kinds of activities, tools, products and applications arising from SynBio research?

The existing risk assessment methodologies, in particular for GMOs and chemicals, are applicable; however, several SynBio developments such as combining genetic parts and the emergence of new properties due to interactions (genetic parts libraries), combinations of chemical and biological assessments (protocells), interactions between xenobiological and natural organisms (xenobiology), and the acceleration of GM processes will require improving existing methodology.

Question 6: If existing methodologies are not appropriate to assess the potential risks associated with activities related to and products arising from SynBio research, how should existing methodologies be adapted and/or completed?

Though present risk assessment methodologies are appropriate for assessing potential risks of SynBio activities and products, the SCs suggest several improvements to ensure continued safety protection proportionate to risk, while enabling scientific and technological advances in the field of SynBio. These improvements include, 1) support the characterisation of the function of biological parts and the development of computational tools to predict emergent properties of SynBio organisms, 2) streamline and standardise the methods for submitting genetic modification data and genetic parts information to risk assessors, 3) encourage the use of GMOs with a proven safety record as acceptable comparators for risk assessment, 4) aim to ensure that risk assessment methods advance in parallel with SynBio advances, and 5) support the sharing of relevant information about specific parts, devices and systems with risk assessors.

Question 7: How, when, and to what extent can safety (safety locks) be inherently built into products of SynBio?

Currently available safety locks used in genetic engineering such as genetic safeguards (e.g., auxotrophy and kill switches) are not yet sufficiently reliable for SynBio. Notably, SynBio approaches that provide additional safety levels, such as genetic firewalls may improve containment compared with classical genetic engineering. However, no single technology solves all biosafety risks and many new approaches will be necessary.

Question 8: The SCENIHR, SCHER, SCCS are asked to draw the blue print of a general procedure/strategy for designing inherently safe applications of SynBio.

A blue print of a general strategy for designing inherently safe applications of SynBio is demanding, because of the stochastic and probabilistic character of the underlying biochemical SynBio processes. General biocontainment approaches are based on 1) physical containment, 2) inhibition of uptake, 3) incorrect translation, 4) inability to replicate, 5) absence of host immunity and 6) endogenous toxicity. For instance, genetic safeguards such as auxotrophy and kill switches are not sufficiently reliable/robust for field release of engineered bacteria, because of mutation and positive selection pressure for mutants that may lead them to escape safeguards. The SCs recommend a clear strategy for the analysis, development, testing and prototyping of applications based on new forms of biocontainment and additional layers of containment using orthogonal systems.

9.4. Annex IV Key technologies with potential impact on risks to the environment

Short description with reference to Opinion I

Genetic parts: SynBio library construction and parts characterisation may increase the frequency of use of uncharacterised components, and/or the diversity of biological functions. The function of these systems may be "emergent," i.e. they arise from the interactions of the parts with each other. Emergent functions may include conditional, time-varying and non-linear (non-proportional) behaviours (Guet *et al.*, 2002). The current Directives 2001/18/EC and 2009/41/EC for risk assessment consider these emergent properties by requiring an assessment of the proposed or realised GMM/GMO, in addition to an assessment of the properties of component parts. Notably, the emergent properties may present new challenges in predicting or testing for risks and in the identification of appropriate comparator organisms.

Minimal cells and designer chassis: Minimising the number of components required to support biological synthesis from synthetic DNA circuits or genomes may also simplify control of the function(s).

Protocells: Currently, protocells are non-living vesicles and will likely be confined to the laboratory for the near to medium-term. Although the objective is for such cells to replicate, it is not yet possible. Therefore, dispersion is not possible because of the lack of cell viability. Risks related to protocell research are no higher than the risks in biological and chemistry laboratories (Bedau *et al.*, 2009), because the current state-of-the-art research does not create novel, viable artificial cells. In the future, exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Although protocells are not alive, they can be engineered to intimately interact with living cells and enhance overall system functionality. Thus, novel biological functions can be designed without altering the DNA of these target organisms. If autonomous artificial cells are created in the future, the genetic information that controls internal functioning might mutate or be horizontally transferred. Thus, a population of protocells with different genetic information could undergo selection and new protocells could arise (Bedau *et al.*, 2009).

Xenobiology: The use of non-standard biochemical systems in living cells, e.g., XNA, alternative base pairs, etc., has implications for risk assessment and biosafety. (New variants must be tested for risk to human health or the environment and the xenobiological systems may be engineered to allow for improved biocontainment, e.g., the so-called 'genetic firewall' that aims to avoid) the exchange of genetic material through horizontal gene transfer or sexual reproduction between the XB and natural organisms. The assumption is that xeno-systems would not survive due to their custom-made auxotrophy.

DNA synthesis and genome editing: The new technologies for DNA synthesis and genome editing accelerate genetic modification and increase the range and number of modifications that are easily possible. The increased speed of modifications might pose challenges to risk assessment.

Citizen science: While the hazard remains the same, e.g., infection with pathogenic organisms the probability of unintentional harm might increase, because more people are starting to actively work with biological material. However, as long as the citizen science community is well informed and cautious, the overall additional risk increase would be minimal.