Scientific Committee on Health and Environmental Risks

SCHER

Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Scientific Committee on Consumer Safety

SCCS

Opinion on
Synthetic Biology I
Definition

The Scientific Committees adopted this Opinion:

The SCCS at their plenary on 23 September 2014, the SCENIHR at their plenary on 24 September 2014 and the SCHER by written procedure on 25 September 2014

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 the 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 in order to contribute to the relevant issues in close cooperation with other European agencies.

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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 new risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases

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ABSTRACT

This Opinion is the first of a set of three Opinions addressing a mandate on Synthetic Biology (SynBio) from Directorates Health and Consumers (SANCO), Research and Innovation (RTD), Enterprise and 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 (EU), 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 (GMO)?

Over the past decade, new technologies, methods and principles have emerged that enables for faster and easier design and manufacturing of GMOs, which are referred to as Synthetic Biology (SynBio) and 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 Biosafety. 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.

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 which modification was achieved, the number of independent modifications, or the degree of computational design methods used, alone or 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 enables the rapidly advancing nature of GM technologies and adds an important nuance that supports the need for on-going updates of risk assessment methods, which will be addressed in Opinion II.

Keywords: Synthetic biology; biotechnology; bioengineering; genetic engineering; microbiology; molecular biology; Regulatory framework; genetically modified organisms (GMO); definition

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1 BACKGROUND

1.1 General introduction

Synthetic Biology (SynBio) aims to design biological systems that do not exist in nature. Synthetic biologists use engineering principles and re-design existing principles 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 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 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 results in 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 Working Group were focused on the direct implications on plant breeding, synthetic genomics was also considered. The Report from this Working Group was finalised in January 2012 (NTWG, 2012 New techniques working group (2012) Final Report) and the main conclusion was that synthetic genomics / SynBio is a fast-evolving field that differs from previous gene modification techniques. Furthermore, the NTWG Working Group was uncertain whether Directives 2009/41/EC and 2001/18/EC (see Annex V) from the European GMO regulatory framework were the appropriate legislation to cover synthetic genomics and Synbio.

1.3 Scientific background

The EC supports and has supported research on the scientific and societal implications of SynBio via its Framework programmes for Research and Technological Development including the engagement of stakeholders and promotion of exchange of information and knowledge with and within the SynBio community. The multidisciplinary nature and breadth of SynBio makes the assessment of state-of-the-art developments, the nature of foreseen applications and their time to market challenging, but insights are available in the following projects and reports:

A. NEST 2005: European Synthetic Biology. Applying engineering to biology (*Report of a NEST high-level Working Group. Luxembourg: Office for Official Publications of the European Communities. EUR21796*) and SynBio EC FP6 and FP7 projects involving a variety of engineering approaches (e.g. minimal genome, standardisation, gene transcription, cell membrane), and applications (e.g., biocatalytic processes,

diagnostic, drug development delivery, energy, bioremediation) as well as training, regulatory and societal aspects, governance and ethics (see a.o. Annex II for a list of key FP6- and FP7-funded projects).

B. Recommendations from the European Group of Ethics (EGE) outlined in the Opinion on the ethical aspects of SynBio adopted on 17 November 2009 upon request from the EC President for risk assessment including a survey of relevant bio-safety procedures (*EGE (2009) Ethics of Synthetic Biology. European group on ethics in science and new technologies in the European Commission. Opinion No. 25. Brussels*).

C. 5th meeting of Chairs and Secretariats of the EU Commission and Agency Scientific Committees and Panels involved in Risk Assessment, organised by DG SANCO in Brussels on 18-19 November 2009 *(EC, 2010, Meeting Report. Brussels, 17-02-2010)*.

D. SynBio Workshop: "From Science to Governance", organised by DG SANCO on 18-19 March 2010. There is a need for an appropriate risk analysis and a systematic consideration of the relevant safety aspects to facilitate a comprehensive assessment of this new technology (*EC, 2010 From Science to Governance A workshop organised by the European Commission's Directorate-General for Health & Consumers 18-19 March 2010, Brussels*).

E. Information on SynBio techniques, tools and applications published in the general press and in peer-reviewed journals, e.g. a recent announcement of the creation of a bacterial cell controlled by a chemically synthesised genome.

F. The international symposium on “Opportunities and Challenges in the Emerging Field of SynBio” in July 2009 in Washington, DC, under the auspices of the United States National Academies, the Organisation for Economic Co-operation and Development and the Royal Society.

G. Other relevant available scientific information from various stakeholders e.g. European Molecular Biology Organisation.

2 TERMS OF REFERENCE

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

2.1 Scope and definition of the phrase “Synthetic Biology”

1. What is Synthetic Biology 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

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

definition of "Synthetic Biology"? 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?

These questions are part of a set of 11 questions from the EC on SynBio (Annex I). In addition to the above questions on the scope and definition, there are 5 questions on risk assessment methodology and safety aspects and 3 questions on research priorities that will be addressed in future companion Opinions.

Although security issues concerning SynBio are important, the terms of reference pertain exclusively to the safety of SynBio. Therefore, security issues will neither be discussed in this Opinion nor in the two subsequent companion Opinions.

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 and authorities. To facilitate the task of the Committee, the EC contracted two searches of the published literature. The first covered SynBio literature published up to the beginning of 2013 and the second covered papers published afterwards. In addition, a search was conducted of publications by governmental bodies relating to the regulation of GMOs and SynBio. The searches yielded approximately 350 publications. Relevant publications published before February 1st 2014, the closing date for data considered for this Opinion, were identified and critically examined. More recent publications were included if they were considered critical for this Opinion. Not all identified studies were necessarily included in the Opinion. On the contrary, a main task was to evaluate and assess the articles and the scientific weight given to each of them. Only studies that are considered relevant for the task are 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 risk assessment purposes, a weight of evidence approach" (SCENIHR, 2010).

3.2 Key general terms

There are several general terms which are considered to be key to this Opinion and therefore these are explicitly defined here:

Organism: any biological entity capable of replication or of transferring genetic material (Directive 2001/18/EC)

Modern biotechnology: means the application of in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid

into cells or organelles, or fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection (Cartagena Protocol on Biosafety)

Genetic modification: the processes leading to the alteration of the genetic material of an organism in a way that does not occur naturally by mating and/or natural recombination (Directive 2001/18/EC and 2009/41/EC, see Annex V)

Genetically modified (micro-)organism: a (micro-) organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (Directive 2001/18/EC and 2009/41/EC, see Annex V)

Living organisms: any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids (Cartagena Protocol on Biosafety)

Living modified organism: any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (Cartagena Protocol on Biosafety

Genetic engineering in this Opinion refers in general to the techniques/methodologies used for genetic modification. Genetic material is considered to be any physical carrier of information that is inherited by offspring.

3.3 Scope and Definition

3.3.1 Main scientific developments

For risk assessment, the SC will broadly consider recent advances in tools and concepts that currently facilitate and accelerate the generation of GMOs, with a focus on identifying qualitative or quantitative changes in the type or scope of genetic modification that potentially creates new risks and opportunities.

The completed sequence of the human genome in 2001 (Lander et al., 2001; Venter et al., 2001) and the dramatic improvements in genome sequencing technology that followed (Koboldt et al., 2013; Metzker, 2010) changed scientific perception of the possibilities of genetic engineering. Because it is possible to sequence virtually any genome rapidly and at low cost, genetic modification is now done in the context of detailed knowledge of the entire genome. This has inspired a desire to write genetic sequences (Dietz and Panke, 2010; Tian et al., 2009) and led towards the development of new concepts and tools that facilitate and accelerate genetic engineering. SynBio has emerged as a new research area associated with an expansion of the scope and scale of genetic modification. In addition to the development of novel methodologies and applications, it is also vital to appreciate the great scientific importance of synthetic biology in helping to achieve better understanding of natural biological systems (Chen et al., 2012; Cheng and Lu, 2012; Heinemann and Panke, 2006; Keasling, 2012; Khalil and Collins, 2010; Kitney and Freemont, 2012; Liang et al., 2011; Pleiss, 2006).

3.3.1.1 SynBio Concepts

The most notable conceptual development in the area of genetic engineering is the adoption of classical engineering concepts such as standardisation and modularisation

and an attempt to apply these to the engineering of biological systems (Agapakis, 2013; Cheng and Lu, 2012; Heinemann and Panke, 2006). Some concepts are outlined as follows:

A. **Standardisation:** Standardisation is an important classical engineering concept that could influence the efficiency of genetic engineering (Muller and Arndt, 2012). For example, the International Genetically Engineered Machine (iGEM, Annex II) competition, a defining event in the field (Foundation, 2014; Kitney and Freemont, 2012), is built around the concept of BioBricks (Boyle et al., 2012; Shetty et al., 2008), which are based on standardising nucleotide sequences for easier engineering and facilitates the exchange of engineered sequences between research groups. Currently, researchers who are not on an iGEM team rarely use standardised parts from community repositories. Most engineered nucleotide sequences reported in published research do not use standardised cloning sites and are not introduced into the same vector. Standardisation is particularly difficult to achieve for functional characterisation of engineered components and systems (Kwok, 2010). Currently, standards are mostly used internally within research groups or companies.

B. **Modularisation**: This concept is a basic phenomenon of genetic engineering in which genes, protein domains and promoters are modules that can be recombined to generate new functionality (Agapakis and Silver, 2009). This concept closely relates to hierarchical abstraction in which modules (genes, protein domains, promoters, and genetic circuits) may theoretically be used without considering internal molecular functional details. This enables decoupling of design and fabrication, which enables for a division of the engineering process into smaller sub-problems that can be worked on independently. Eventually, the processes may be combined to produce a functioning whole (Endy, 2005). Currently, this is a hypothetical concept (Kwok, 2010).

C. **Orthogonality**: The modularisation of the genetic engineering process can potentially be improved by employing parts and devices made from parts, which are functionally orthogonal to the cellular machinery of the engineered host organism. A common application of this principle is the use of prokaryotic gene regulation systems in eukaryotic organisms, and vice versa (An and Chin, 2009; Stanton et al., 2014; Temme et al., 2012a). More ambitious plans are to use non-DNA-based information carriers, which create artificial genetic systems that function independently orthogonally from the host organism and cannot be read by non-engineered natural organisms (Herdewijn and Marliere, 2009; Schmidt, 2010; Wright et al., 2013).

D. **Refactoring**: The software-engineering concept of refactoring refers to the process of substantial rewriting of existing software code without changing its external behaviour. In genetic engineering, this approach may be applied to the rewriting of genetic information, so that the protein-coding information is maintained, but the sequence is otherwise randomised and all regulatory elements are replaced by specifically designed DNA parts (Chan et al., 2005; Ghosh et al., 2012; Shao et al., 2013; Temme et al., 2012b). The intention is to remove all uncharacterised functional elements and molecular interactions, which might lead to unpredictable system behaviour (Temme et al., 2012b).

3.3.1.2 Synthetic Biology Tools

The SynBio toolbox is evolving dynamically as molecular biology advances and researchers adapt and adopt tools from unrelated fields (Lee et al., 2013; Seo et al., 2013; Tyo et al., 2010; Wang et al., 2013). An overview of the current state-of-the-art SynBio tools is provided in Kahl and Endy, 2013 and summarised below.

A. **Design tools**: Software and bioinformatics tools are widely used in the engineering process. BioCAD tools, analogous to the Computer-Aided Design software used in mechanical engineering, are becoming more sophisticated (Chandran et al., 2009; Lee et al., 2007; Rodrigo and Jaramillo, 2013; Xia et al., 2011), can be directly combined with tools to assist in the gene assembly process (Richardson et al., 2006; Villalobos et al., 2006) and interfaced with robotic machinery that performs the actual cloning and transformation experiments (Densmore and Bhatia, 2013; Slusarczyk et al., 2012). Another major area of research focuses on the development of simulation tools, which enables for the prediction of the behaviour of the engineered system. These tools include metabolic modelling approaches, sometimes based on comprehensive computational descriptions of the stoichiometry of the entire metabolic network (Medema et al., 2012; Mendes et al., 2009) as well as simulators to predict the behaviour of individual molecules such as RNA folding or Ribosomal Binding Site properties (Garcia-Martin et al., 2013; Hofacker and Lorenz, 2014; Salis, 2011). Recent progress in protein engineering established computational methods to identify enzyme targets for optimisation, design and model improved functional proteins and to develop novel enzymes to catalyse any chemical reaction of interest, including those not occurring in natural organisms (Bjelic et al., 2013; Marcheschi et al., 2013; Mills et al., 2013; Procko et al., 2013; Richter et al., 2011).

   DNA databases and registries containing standardised information about strains, genes, plasmids, enzymes, promoters, ribosome binding sites and terminators complement the computational design tools. The parts documented in these databases may be ordered and used in the assembly of new DNA constructs. The best known example of this type of a database is the iGEM Part Registry, containing over 10000 parts, most of which were generated and characterised by student teams in the annual iGEM competition (Muller and Arndt, 2012). American Type Culture Collection (ATCC) and Addgene are other DNA registries widely used in the SynBio community (Kahl and Endy, 2013). The availability of characterised elements that can be used in the systems design process is not limited to individual parts, such as genes, but also extends to functional devices, such as regulatory circuits of defined behaviour, ranging from simple switches (Gardner et al., 2000) to complex timing devices (Weber et al., 2007), counters (Friedland et al., 2009), oscillators (Stricker et al., 2008) and logical gates (Bonnet et al., 2013; Iyer et al., 2013; Qi et al., 2013).

B. **Construction tools:** Arguably the most important tool for advanced genetic engineering is accelerated DNA synthesis technology (Gibson et al., 2008; Gibson et al., 2010; Hughes et al., 2011), which needs to be combined with advanced methods for the assembly of the synthesised DNA parts into larger functional units (Esvelt and Wang, 2013; Merryman and Gibson, 2012; Miklos et al., 2012; Tsvetanova et al., 2011). For smaller DNA constructs, methods such as BioBrick cloning and Gateway cloning are often employed (Ellis et al., 2011), while approaches such as the Gibson assembly method and de novo assembly are commonly used for larger constructs, up

to the whole-genome scale (Merryman and Gibson, 2012). The genome synthesis and assembly methods are complemented by a growing set of genome-editing tools, which help to modify existing DNA sequences more quickly and reliably (Esvelt and Wang, 2013), often by exploiting highly parallelised approaches that enable the near-simultaneous evaluation of large libraries of engineered systems (Wang and Church, 2011). Widely used tools for this purpose include: directed evolution for the selection of proteins or nucleic acids with desired new properties; Multiplex Automated Genome Engineering (MAGE) for the rapid generation of libraries of targeted mutations; Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) for easy and highly specific gene editing and the construction of programmable transcription factors; and Transcription activator-like effector nucleases (TALENs), as well as zinc finger domains, for unlimited design of novel restriction enzymes with engineered binding site specificity (Esvelt and Wang, 2013).

C. **Diagnostic tools**: The engineering of complex biological systems critically depends on the availability of diagnostic tools to characterise the phenotype and functionality of the engineered organism, shared with the classical areas of molecular biology. These include a wide range of microscopy imaging techniques (including optical, electron and atomic force microscopy) and post-genomic molecular profiling methods for quantifying various types of complex biomolecules (transcriptomics, proteomics, metabolomics (Ellis and Goodacre, 2012; Nguyen et al., 2012; Sagt, 2013).

3.3.1.3 Synthetic Biology Research Areas

A. **Synthetic genomics and DNA synthesis:** The foundation for all recent advances in genetic engineering is the increased ability to chemically synthesise DNA and to assemble it into constructs that can be introduced into living organisms. The resulting enhanced ability to write DNA is the key to implementing most of the concepts described above. It is currently possible to chemically synthesise and assemble DNA on the scale of entire microbial genomes (Anemaet et al., 2010; Gibson et al., 2008; Gibson et al., 2010; Lartigue et al., 2009) and to transfer these synthetic genomes between, for example, yeast and bacteria (Benders et al., 2010). To date, genome-scale DNA synthesis was mostly used for “chemical copying” of natural genomes (Gibson et al., 2008). However, a future application will be the synthesis of minimised genomes stripped of redundant genetic information “junk DNA” and genes that are unnecessary for the intended function (Murtas, 2009; Stano and Luisi, 2013; Zhang et al., 2010).

B. **Metabolic Engineering:** Application areas of SynBio include a wide range of fields, such as chemical synthesis, plant trait engineering, tissue engineering, gene therapies, and novel medicines. Many of the current cutting-edge research applications of the extended scope and scale of genetic modifications are in the bio-based production of fuels, chemicals and plastics (Frasch et al., 2013; Kung et al., 2012; Seo et al., 2013; Siddiqui et al., 2012; Stephanopoulos, 2012; Tippmann et al., 2013). Compared to earlier gene-by-gene approaches and genome-scale metabolic engineering, SynBio has the potential to transform the costs and time involved in making new strains for bio-based production (Sandoval et al., 2012). An early example is the production of the anti-malarial drug precursor artemisinic acid in yeast and bacteria instead of the natural producer, the sweet wormwood plant (Anthony et al., 2009; Ro et al., 2006; Tsuruta et al., 2009; Westfall et al., 2012).

Optimal production required the combination of several approaches: 1) the utilisation of genes from a variety of different organisms, 2) the engineering of the regulatory circuitry to fine-tune enzyme levels in the biosynthetic pathway as well as in competing reactions, and 3) the use of artificial protein scaffolds to optimise the stoichiometry of the biosynthetic enzymes (Anthony et al., 2009; Keasling, 2012). A number of chemicals produced by microbes that were engineered using this approach have recently entered the market including biofuels and high-value chemicals such as drugs, flavours and fragrances (Hayden, 2014; Project, 2012). One alternative for the future of metabolic engineering is based on cell-free *in vitro* systems. Recently, attention has turned to designing cell-free factories for making chemicals (Swartz, 2006). In the future, cell-free factories may be capable of more resource-efficient metabolism, because resources are not diverted to sustaining the life of the cell (Hodgman and Jewett, 2012). This might lead to the development of sustainable production systems for biofuels and bulk chemicals.

C. **Orthogonal biosystems / xenobiology:** Most engineering activities in SynBio re-use existing biochemical components, such as DNA as the main information carrier and the 20 canonical amino acids as the main protein constituents. In recent years, a branch of SynBio has successfully started to design alternative biochemical components for bioengineering. Most of the work in this field focuses on exploiting nucleic acid analogues (Xeno Nucleic Acids) as orthogonal information carriers unusable by natural biological systems (Herdewijn and Marliere, 2009; Pinheiro et al., 2012). In addition, biologists have started to change the genetic code by reprogramming the codon-amino acid table (Lajoie et al., 2013) and expand the repertoire beyond the canonical 20 amino acids (Budisa, 2004; Wang et al., 2001). The use of novel non-canonical amino acids will increase the biochemical functionality of proteins (Voloshchuk and Montclare, 2010). Xenobiology may offer new opportunities for the development of novel biocontainment systems through the implementation of “genetic firewalls” (Moe-Behrens et al., 2013; Schmidt, 2010; Wright et al., 2013)**.**

D. **Protocells**: Most work in SynBio starts with some pre-existing natural living system and then re-engineers it for specific desired purposes. Another approach to engineering novel biological systems works strictly from the ‘‘bottom up’’ and attempts to construct new simple forms of living systems, using chemical and physical processes and employing as raw ingredients only materials that were never alive (Bedau et al., 2009). Currently, the systems constructed by bottom up approaches are not alive, but are chemical vesicles, called ‘‘protocells’’ (Rasmussen, 2009). The long-term ambition of this line of research is to produce protocells that are sufficiently functionalised, so that they may be used as containers or chassis into which synthetic heritable material could be introduced resulting in novel living, self-replicating organisms (Danchin, 2009). Some basic systems were developed, including the demonstration of chemical copying of RNA templates inside protocells (Adamala and Szostak, 2013; Blain and Szostak, 2014), but more sophisticated protocells with complex functionalities (especially the capacity of robust self-replication) are not yet available. Although protocells (just like “naked” DNA molecules) are not alive *per se*, they represent important initial steps towards the synthesis of living organisms. Protocells can be designed to enhance the functionality of living cells, e.g. by extending the capacity to detect and interact with the environment. The potential of protocells as precursors of fully synthetic cells and

their deployment to modify the capabilities of living organisms clearly qualify them as part of synthetic biology research.

3.3.2 Regulatory aspects (GMO-regulation, Convention on Biodiversity)

For the purposes of this Opinion, this section presents a brief overview of the key principles of relevant existing regulatory frameworks for SynBio aimed at protecting human health and environment.

3.3.2.1 Regulatory aspects in the European Union

Although SynBio is relatively a new field, the existing regulations applicable to biological, chemical or genetic modification research and products are also applicable to SynBio research, applications and products (Annex IV). In particular, the safety and regulatory aspects for SynBio are considered in light of the current EU GMO regulatory framework (embodied by EU Directives 2001/18/EC regulating deliberate release, and 2009/41/EC regulating contained use).

Directive 2009/41/EC, Article 4(3) encompasses a classification of contained uses or activities involving GMMs into 4 classes depending on their potential risk to health and the environment:

The procedure for determining risk class is outlined in Annex III of Directive 2009/41/EC with Annex IV of this Directive presenting normal minimum requirements and measures necessary for each level of containment.

While Directive 2009/41/EC only covers the contained use of GMMs, specific European Member States, such as Belgium, implemented the Directive into their national legislation by broadening the scope to include GMOs and pathogenic organisms for humans, animals and plants. In Switzerland, the Directive on contained use of GMMs served as basis for the set-up of national legislation covering work with biological agents.

The second regulation governing GMOs is Directive 2001/18/EC on the deliberate release of GMOs into the environment. To date, this regulation has been predominately applied to regulate the field trials, cultivation and commercial release of GM plants, although it governs all GMOs.

The EU GMO regulatory framework relies on the tools and approaches underlying, amongst others, 1) recombinant DNA techniques, 2) the direct introduction of heritable material into an organism and 3) cell fusion or hybridisation techniques (Annex I, Part A of Directive 2009/41/EC and Annex I A Part I of Directive 2001/18/EC, see Annex V of this opinion). Therefore, risk assessment takes into account risks posed by the tools and approaches (process) used to generate GMOs. However, there is currently debate on whether process-based analysis should be applied for the regulatory oversight of certain novel techniques for genetic modification, as exemplified by the debate on the new plant breeding techniques (NPBT)[2]. One of the reasons for the debate is that process-based

[2]These techniques have the potential to make the breeding process faster while lowering the production costs. In some cases, they enable for site-specific and targeted changes in the genome based on genetic modification techniques or avoid the stable introduction of transgenes, making them also indistinguishable from plants obtained by conventional breeding. Therefore, plants developed by NPBT that do not contain recombinant DNA in their genome are challenging the current GMO legislation. The uncertainty of the regulatory status of plants developed by NPBTs could have an impact on innovation, because it is difficult for a plant-breeder to decide if he/she should invest his/her efforts in a project using one of these techniques. While conventional breeding techniques present relatively low registration costs, transgenic plants regulated under the GMO jurisdiction

triggers for regulatory oversight might rapidly outgrow new biotechnology-based tools and approaches. The developments in plant breeding and the uncertainty of the regulatory status of NPBT in Europe are included in several reports and statements arguing for a more flexible and product-based approach of the legislation (EASAC, 2013; Heap, 2013; Morris and Spillaine, 2008; Podevin et al., 2012). These considerations may also apply to the regulatory oversight of organisms generated by SynBio.

3.3.2.2 Official statements and recommendations on SynBio in Europe:

In the EU, a number of governmental bodies, national academies and transnational networks issued statements and recommendations on safety and regulatory aspects of SynBio (new Annex) and expressed a set of common questions on whether Synbio can be considered in the EU GM regulatory framework:

- Zentrale Kommission für die Biologische Sicherheit (ZKBS, Central Committee on Biological Safety, Germany[3])
- Swiss Academy of Technical Sciences (www.geneticresearch.ch/f/themen/Synthetic_Biology/index.php)
- The Royal Netherlands Academy of Arts and Sciences, together with the Health Council of the Netherlands and the Advisory Council on Health Research (Health Council of the Netherlands et al. 2008[4])
- German Academy of Sciences Leopoldina and German Academy of Science and Engineering and the German Research Foundation (DFG 2009[5])
- The Netherlands Commission on Genetic Modification (COGEM 2013[6])
- The Royal Academy of Engineering in the UK (Royal Academy of Engineering 2009[7])
- Health and Safety Executive (HSE 2012[8]), which presented a review of the technology and current and future needs from the regulatory framework in Great Britain

were associated with high registration costs and extensive risk assessment procedures. For example, procedures in Europe include the evaluation of substantial differences between GM crops and their non-GM counterparts, molecular characterisation, toxicity and allergenicity studies and the assessment of the environmental impacts and unintended effects (EC, JRC, 2011)

[3]The Zentrale Kommission für die Biologische Sicherheit (ZKBS, Central Committee on Biological Safety, Germany (2012) http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/01_Allgemeine_Stellungnahmen_deutsch/01_allgemeine_Themen/Synthetische_Biologie.pdf?__blob=publicationFile&v=3

[4]Royal Netherlands Academy of Arts and Sciences (2007) A Code of Conduct for Biosecurity. Report by the Biosecurity Working Group. pp. 1-44. https://www.knaw.nl/nl/actueel/publicaties/a-code-of-conduct-for-biosecurity. ISBN 987-90-6984-535-7

[5]Deutsche Forschungsgemeinschaft (2009) Synthetische Biologie: Stellungnahme. ed. Deutsche Forschungsgemeinschaft. ISBN 978-3-527-32791-1

[6]The Netherlands Commission on Genetic Modification (COGEM), 2013. Synthetic Biology – Update 2013. Anticipating developments in synthetic biology. COGEM Topic Report CGM/130117-01. http://www.cogem.net/index.cfm/en/publications/publicatie/synthetic-biology-update-2013

[7]Royal Academy of Engineering (2009) Synthetic Biology: scope, applications and implications. https://www.raeng.org.uk/societygov/policy/current_issues/synthetic_biology/default.htm. ISBN: 1-903496-44-6

[8]Health and Safety Executive (HSE) (2012). Synthetic Biology. A review of the technology, and current and future needs from the regulatory framework in Great Britain. RR944 research reports. http://www.hse.gov.uk/research/rrhtm/rr944.htm

- European Group on Ethics in Science and New Technologies, who in 2009 presented a comprehensive Opinion with several recommendations on the ethical, legal and social implications of SynBio (EU, 2010[9])

- European Academies Science Advisory Council formed by the national science academies of the EU Member States, who issued a report in 2010 on scientific opportunities and good governance in the field of SynBio (EASAC 2010[10])

- The ERASynBio project, a European Research Area Network funded through the EC seventh Framework program, published a strategic vision) for the responsible development of development of synthetic biology (ERASynBio, 2014[11])

- The Biosafety and Biotechnology Unit (Scientific Institute of Public Health, Belgium) issued two documents: Pauwels et al., 2012 and 2013.

In conclusion, the statements and recommendations listed above express a set of common questions on whether SynBio can be considered in the EU GM regulatory framework, which are listed below:

- Does SynBio present any health and safety risks that are not covered by existing legislation?

- Could current applications of SynBio be covered by GM regulatory framework?

- What are the gaps in current risk assessment procedures in the EU and how should these be addressed in light of the advent of novel products developed using methods of SynBio?

- Which developments in SynBio challenge the GM regulatory framework for being fit for SynBio applications?

- The precautionary principle is key to the GM regulatory framework. How will it be possible to address this concept for SynBio applications?

In response to these questions, the main conclusions emerging from the statements and recommendations of the EU governmental bodies and national academies are:

A. There is a consensus that management and regulation of SynBio work should go through a risk assessment procedure.

B. It is not clear how principles underlying the current GMO regulatory framework, such as the case-by-case risk assessment, the comparative approach, the step-by-step process and the precautionary principle, will be used for SynBio. The case-by-case risk assessment implies that the required information may vary in nature and level of detail from case to case, depending on the living modified organism, trait(s), its intended use and the potential effect on the environment. Therefore Directive 2001/18/EC specifies that each GMO should be independently subjected to a risk

[9]European Union (2010). European group on ethics in science and new technologies to the European Commission Ethics of synthetic biology No 25 ISBN 978-92-79-13829-4 doi: 10.2796/10789 European Union, Rapporteurs: Rafael Capurro, Julian Kinderlerer, Paula Martinho da Silva and Pere Puigdomenech Rosell

[10]European Academies Science Advisory Council (EASAC) (2010) Realising European potential in synthetic biology: scientific opportunities and good governance. European Academies Science Advisory Council. ISBN: 978-3-8047-2866-0

[11]European Research Area Network for the development and coordination of synthetic biology in Europe. https://www.erasynbio.eu/lw_resource/datapool/_items/item_58/erasynbiostrategicvision.pdf

assessment prior to its release. The comparative approach is a commonly applied risk assessment methodology for GMOs whereby risk is considered in the context of the risks posed by the non-modified recipients or parental organisms, in the potential and likely receiving environment (Annex III of the Cartagena Protocol on Biosafety (CPB)). The step-by-step process is a concept that guides the introduction of GMOs into the environment. It involves the gradual reduction of the containment of GMOs and an increase of the scale of release, step-by-step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken (Directive 2001/18/EC). The precautionary principle[12] addresses uncertainty as an integral part in risk analysis. It is a key principle of the EU GMO regulatory framework and is even more robustly anchored in the CPB.

C. For current and short-term SynBio developments, risk assessment criteria, methodology and risk management systems established for GMOs and pathogens provide a good basis for addressing potential risks. When well-defined pieces of hereditary material with known function are used according to a pre-determined plan, sufficient knowledge is available to adequately assess and manage the activities with synthetic organisms. In addition, depending on the category and scope of the product, a SynBio product may fall within the scope of specific regulations (Annex IV), which in some cases may also imply a characterisation and safety assessment.

D. Current GMO risk assessment requirements and approaches remain applicable. Short-term SynBio applications might be encompassed by the current GMO definitions in EU GMO legislation. However, some SynBio sub-fields and resulting SynBio products may or may not be covered by existing GMO/GMM legislation. For example, GMO Directives apply to ‘any biological entity capable of replication or of transferring genetic material‘. Synbio systems such as protocells are therefore not (yet) considered as living organisms. Hereditary or genetic material can occur in the form of DNA, but is not restricted to it. For example xeno nucleic acids such as HNA (hexose nucleic acid) or TNA (threose nucleic acid) can also be seen as hereditary material. Even non-nucleic acid molecules can contain hereditary information that is transferred to the next generation. Thus XNA is included in the current EC GMO regulation.

The SC also notes that problem formulation is a critical phase of any risk assessment process regardless of the type of stressor. It provides the context for risk characterisation. Problem formulation, e.g., as defined for chemicals in a WHO/IPCS publication (Meek et al., 2013), includes risk management scope and goals in relation to relevant exposure scenarios, level of uncertainty and risk that is considered acceptable, analysis plan and information needs. A wide range of legislation applies to the main types of materials covered in the environmental risk assessment, namely chemicals, biological products and GMOs. Specific guidance is often available for each piece of legislation. For example, for the deliberate release of GM plants, guidelines were issued based on the principles outlined in Directive 2001/18/EC (EFSA Panel on Genetically Modified Organisms; Guidance on the environmental risk assessment of genetically modified plants[13]).

[12]United Nations, “Rio Declaration on Environment and Development,” Rio de Janeiro, 1992.‘In order to protect the environment, the precautionary approach shall be widely applied by States according to their capability’.
[13]doi:10.2903/j.efsa.2010.1879

Environmental risk assessment covers the risk to all ecosystems, including humans, upon deliberate release in the environment of chemicals and biological products as well as GMOs. The term environmental risk assessment does not normally cover the risks to individuals or the general public at large from consumer products or from exposure in the work place, where other specific legislation applies (Annex IV).

Although protection of the environment and human health are universally required, consideration of animal health and also socio-economic and cultural impacts are highly contextual and hence not universally regulated. What is considered an adverse effect as well as an "acceptable risk" will depend on what is to be protected, where to protect it, and over what time period. The importance of specifying protection goals and their assessment endpoints should be emphasised, because risk assessors need to translate them into specific protection goals to facilitate a structured approach for identifying potential risks and scientific uncertainties (problem formulation). A common understanding of protection goals and their implementation into clear endpoints is crucial.

The growing concern over the burden posed by the complexity of the risk analysis process and decision-making in the field of GMOs for large-scale dissemination further emphasises the importance of setting up clear policy objectives. The amount of effort and detail required in assessing each risk can vary and should be proportionate to priority and complexity. EFSA is currently exploring the possibility of developing a harmonised framework to specify protection goals for application to an agro-landscape regardless of the product or organism that is being assessed (EFSA's 19th Scientific Colloquium – entitled "Biodiversity as a protection goal in environmental risk assessment for EU agro-systems").

3.3.2.3 Regulatory aspects in the United States

In the USA, the dominant idea is that the existing policy and regulatory framework for biotechnology applies, with minor adaptations, to synthetic organisms. Laboratory research in USA is the remit of the National Institute of Public Health. Their biosafety system for risk assessment and categorisation of biological risk served as a reference document for the development of legislation and guidelines worldwide and encompasses the use of Biosafety levels (BSL) 1 to 4. For synthetic nucleic acids, the NIH Recombinant DNA Advisory Committee concluded that in most cases, biosafety risks are comparable to recombinant DNA research and that the current risk assessment framework can be used to evaluate synthetically produced nucleic acids with attention to the unique aspects of this technology. To provide principles and procedures for risk assessment and management of research involving synthetic nucleic acids, the NIH Guidelines for research involving recombinant DNA molecules was adapted to specifically cover synthetic nucleic acid molecules (NIH guidelines for research involving recombinant or synthetic nucleic acid molecules[14]).

Of note:

a) Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g. a toxin or a pharmacologically active agent) are regulated in the same way as their natural DNA counterpart.

[14]http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm

b) If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempted from the NIH Guidelines; Exempted from NIH guidelines are those synthetic nucleic acids that: can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication nor contain elements known to interact with either DNA or RNA polymerase), are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 ng per kg body weight.

In contrast to the EU GMO regulatory framework, no specific legislation was dedicated to the regulation of organisms derived from biotechnology. For the assessment and regulation of biotechnology products, including their intended environmental releases of organisms, a coordinated framework was put in place by the Environmental Protection Agency (EPA), the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) and the Food and Drug Administration (FDA). This coordinated framework is considered appropriate for regulating most of the organisms obtained by near-term SynBio applications. However, unlike plants obtained by older genetic modification techniques, the engineering of organisms without the use of a (component of a) plant pest would shift them out of the regulatory review of APHIS. It is also expected that EPA regulators will face an increased influx of genetically engineered microbes intended for commercial use for which the risk assessment will pose a greater challenge (JCVI, 2014[15]).

In 2010, the US Presidential Commission for the Study of Bioethical Issues (PCSBI, 2010) published a report recommending the adoption of a system of "prudent vigilance that carefully monitors, identifies and mitigates potential harms over time". However, the term "prudent vigilance' is not clearly defined. Five ethical principles and 18 recommendations were highlighted in this report, including mandatory ethics training for engineers working in the area, identification of gaps in the risk assessment practices, adoption of measures that would limit the survival/lifespan of synthetic organisms in the event of inadvertent/accidental release in the environment and continuous assessment of specific security and safety risks of SynBio research activities in both institutional and non-institutional settings as the field progresses.

3.3.2.4 Regulatory framework of Canada: an example of product-based regulation

In Canada, products derived through biotechnology are treated as any other novel product. This means that regulation is triggered by the novel trait of the product, novel feeds and novel foods and not by the process via which the trait is introduced. The risk assessment is basically a science-based and product-based approach. Health Canada is the federal government department that regulates health products, food products and environmental/industrial products by assessing and managing the risks associated with their use. An overview of the regulatory framework for biotechnology can be found on the pages of Health Canada:

- Health & environment: http://www.hc-sc.gc.ca/sr-sr/biotech/environ/index-eng.php

[15]J. Craig Venter Institute (JCVI) (2014). Synthetic Biology and the U.S. Biotechnology regulatory system: Challenges and options. http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-biology-and-the-us-regulatory-system/full-report.pdf

- Health products: http://www.hc-sc.gc.ca/sr-sr/biotech/health-prod-sante/index-eng.php

3.3.2.5 Official views in China[16]

There is no dedicated regulation established in China to guide research activities in SynBio. The current research governance model in China is based on scientifically informed, evidence-based approaches that are, in general, thought to be sufficient to cope with the current state-of-the-art of SynBio research. Most of these regulations were drawn based upon international guidance, such as the International Committee on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use Good Clinical Practice Guidelines, the CPB for Living Modified Organisms (LMOs) and the World Health Organisation (WHO) handbook for laboratory biosafety (WHO, 1984). The Ministry of Science and Technology (MOST) of the Government of the People's Republic of China, formerly called the State Science and Technology Commission, is the most important national body to develop regulations in science and technology policy. The guidelines promulgated by MOST have a nationwide scope. GMOs for agricultural purposes, such as transgenic crops, are regulated under legislation specifying the biosafety management, trading labelling of agricultural products derived from GMOs.

Current SynBio-related research involving pathogenic microbes, including the microorganism itself or the related medical application is covered by a dedicated guideline for laboratory safety of infectious agents[17]. The law Methods for the Biosafety Environmental Management of Pathogenic Microbiology Laboratories[18], issued in 2006, specifies that biosafety laboratories are classified in four levels (BSL-1, 2, 3, 4); which means that research involving highly pathogenic microorganisms can only be conducted in certified BSL-3 and BSL-4 laboratories. Progress was made in improving biosafety standards, standards for containment and guidelines for facilities, however, the implementation of biosafety rules varies depending on the setting in which research occurs.

Some SynBio projects (Pei, 2012; Pei et al., 2011; Schmidt and Pei, 2011) were assessed and funded by the programmes initiated by the Law of the People's Republic of China on Science and Technology Progress[19], which specifies that the State should lay out guidance for scientific and technological research and development and should establish a modernised scientific and technological research and development system, in accordance with the demands of economic construction and scientific and technological progress. There is currently a search for establishing a framework clarifying responsibility of each involved administration agency to avoid redundancy and/or over administration.

3.3.2.6 Regulatory aspects in Latin America and the Caribbean (LAC) Region

The adoption of rDNA biotechnology in the LAC region has increased in recent years (OECD, 2009). Additionally, there is an emerging involvement of researchers conducting

[16]Adapted from Pei et al, 2012.
[17]http://biosafety.sysu.edu.cn/administer/national/200804/79.html, http://xn.sdmyxy.cn:8013/Article/ShowArticle.asp?ArticleID=20
[18]http://www.sepa.gov.cn/info/gw/juling/200603/t20060308_74730.htm
[19]http://www.asianlii.org/cgi-in/disp.pl/cn/legis/cen/laws/cotscototsmalposatd1146/cotscototsmalposatd1146.html?stem=0&synonyms=0&query=biology

work in SynBio from Latin American countries, particularly from Brazil, Mexico and Argentina (Oldham et al., 2012). However, there is no dedicated regulation established to guide SynBio research activities. Most of the LAC countries are parties of the CPB and many of them have developed biosafety frameworks, but only half of them have operational biosafety regulatory systems in place (Araya-Quesada et al., 2012). Countries with an operational regulatory system in place do not necessarily have the same experience due to differing interests in biotechnology research and applications. Countries with the most biosafety regulatory expertise in the field of GMO management and authorisation processes are Argentina, Brazil, Chile, Costa Rica, Colombia, Honduras, Mexico and Uruguay.

3.3.2.7 Views and initiatives at the international level

There is a need for a common approach for SynBio regulation for which there are several initiatives including the Joint Conference of the OECD, the UK Royal Society and the US National Academies of Science on ''Opportunities and Challenges in the Emerging Field of SynBio'' held in July 2009 in Washington DC (OECD, 2009). Additionally, an international forum for risk assessment and policy debates on the governance of SynBio took place under the provisions of the United Nations Convention on Biological Diversity (CBD)[20]. The aims of CBD were to establish protocols to address three objectives including the conservation of biodiversity, sustainable use of biodiversity[21] and the fair and equitable sharing of benefits arising from the utilisation of genetic resources[22]. The Cartagena Protocol on Biosafety (CPB)[23] to the Convention on Biological Diversity regulates international trade in genetically engineered products and establishes an advanced informed agreement procedure, based on a risk assessment, to enable making informed decisions on whether to accept shipments of LMOs. It is grounded in a relatively robust application of the precautionary approach. Currently, 167 parties participate in the CPB, including China, Brazil, India and the EU and its 28 Member States, but not potentially important international players in SynBio such as Australia, Russia, Argentina and Canada and the USA, which is one of only four countries worldwide besides Andorra, South-Sudan and the Holy See that are not party to the CBD and where significant research and development in SynBio is taking place.

In 2012, a report of the Ad Hoc Technical Expert Group on risk assessment and risk management under the CPB (UNEP/CBD/BS/COP-MOP/6/INF/10) included a list of topics for possible development of additional guidance for risk assessment. One of the potential topics was LMOs produced through SynBio. Currently, however, there is no additional guidance for risk assessment. More recently, preparatory work on SynBio was done by the Executive Secretary of the CBD with a view to enabling the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA) to consider this work at their 18th meeting (SBSTTA 18, June 2014, Montreal), prior to the 12th meeting of the

[20] http://www.cbd.int/emerging/

[21] The *CPB to the Convention on Biological Diversity* aims to ensure the safe handling, transport and use of LMOs resulting from modern biotechnology that may have adverse effects on biological diversity, taking also into account risks to human health. It establishes an advanced informed agreement procedure to provide countries with a basis for making informed decisions on whether to accept shipments of LMOs meeting the above criteria.

[22] The *Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress* to the CPB establishes international rules and procedures on liability and redress relating to living modified organisms

[23] *Nagoya Protocol (NP) on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation* aims at sharing the benefits arising from the utilisation of genetic resources in a fair and equitable way, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies

Conference of the Parties (COP12). Indeed, in its decision XI/11[24], the Conference of the Parties (COP) notes '*based on the precautionary approach, the need to consider the potential positive and negative impacts of components, organisms and products resulting from SynBio techniques on the conservation and sustainable use of biodiversity'* and also '*recognises the development of technologies associated with synthetic life, cells or genomes, and the scientific uncertainties of their potential impact on the conservation and sustainable use of biological diversity and urges Parties and invites other Governments to take a precautionary approach[25]'.* The preparatory work encompasses two notes[26] which compile relevant information on components, organisms and products resulting from SynBio techniques that may have impact on the conservation and sustainable use of biological diversity and associated social, economic and cultural considerations.

The Nagoya Protocol does not explicitly mention one of the synthetic biology research areas described in (3.3.1.3) and for some terms of the Nagoya Protocol , such as for "the utilization of genetic resources"[27], the scope of access and benefit-sharing obligations needs further clarification. For example, the term "functional unit of heredity" is unclear in the NP of CBD as the SynBio focus moves away from individual full gene sequence towards using parts of genes as well as the full genome and proteome. Furthermore, it is unclear whether the term "genetic material" also encompasses digital information due to the increasing use of transfers of digital information.

There is great interest in the potential implications of SynBio based on the three objectives of the CBD: 1) the conservation of biodiversity, 2) the sustainable use of biodiversity, and 3) the fair and equitable sharing of benefits arising from the utilisation of genetic resources. Hence, the SBSTTA and the Parties to the Convention are likely to focus on the potential implications of the field release of synthetic organisms, cells or genomes into the environment.

3.3.2.8 Other regulations, guidelines, recommendations or provisions relevant to SynBio

SynBio tools have prompted considerations on regulatory oversight of the use of infectious agents or toxins, dual-use, biosecurity (also under the umbrella of the Biological and Toxin Weapons Convention), social-economic considerations, initiatives undertaken by the Do-It-Yourself (DIY) community, options for self-governance, the development of a code of conduct for research on synthetic microorganisms, public participation and recommendations for open dialogue with different stakeholders. These matters are reviewed in a variety of recent scientific publications (Bar-Yam et al., 2012; de Lorenzo, 2010; EASAC, 2013; Forschungsgameinschaft, 2009; OECD, 2014; Oldham et al., 2012; Pauwels K, 2012; Schmidt, 2010; Zhang et al., 2011). These aspects will not be addressed in this Opinion (see Annex IV for a list of relevant regulatory frameworks).

[24] http://www.cbd.int/decision/cop/default.shtml?id=13172
[25] In accordance with the preamble of the Convention and with Article 14: http://www.cbd.int/convention/articles/?a=cbd-14, when addressing threats of significant reduction or loss of biological diversity posed by organisms, components and products resulting from synthetic biology, in accordance with domestic legislation and other relevant international obligations.
[26] http://www.cbd.int/emerging/
[27] The "utilization of genetic resources" is defined as conducting research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology.

3.3.3 Elements of a definition (based on inventory)

3.3.3.1 Scope and definition of the phrase "SynBio"

Advances in GM technologies and concepts are expanding the scope and scale of possible genetic modifications, which translates to new approaches for GMO design and manufacture. SynBio refers to this emerging collection of technologies, methods and principles (Chen et al., 2012; Cheng and Lu, 2012; Heinemann and Panke, 2006; Keasling, 2012; Khalil and Collins, 2010; Kitney and Freemont, 2012; Liang et al., 2011; Pleiss, 2006). This concept of SynBio as an extension of GM will be justified and developed further in this Opinion and will be a starting point for Opinion II on risk assessment approaches and Opinion III on research needs.

In developing a suitable operational definition, it is essential to identify, as far as possible, the purpose of the definition and its likely subsequent applications. From a purely risk assessment perspective, the principal purpose of defining SynBio is to assist the identification of processes or products that, because of their nature, scale and/or application, might require a substantial change from the current risk assessment procedures. However, deciding upon a precise definition for SynBio is a challenging task, because it is a rapidly expanding science in which new processes and products may be introduced and derived that are not currently envisaged.

The challenge, therefore, is to provide a definition that is;

- of practical value for the purpose of risk assessment
- is sufficiently broad to enable for further developments in the field

Existing definitions of SynBio (Annex III) emphasise conceptual aspects such as rational design, standardisation, modularisation and related engineering concepts as the main drivers for accelerated and facilitated GMO design, manufacture and exploitation. However, for risk assessment purposes, the SC needs to provide an operational definition derived from a working understanding of SynBio as a collection of conceptual and technological advances (described in Section 3.2). Thus, the aim is to enable faster and easier design and manufacturing of GMOs[28], while responsibly addressing societal challenges in the areas of health, energy and food security. For an operational definition, the SC considers it necessary to focus on actual activities, applications and products of SynBio, instead of on abstract concepts and metaphors.

In most cases tangible/measureable parameters, alone or in combination, excluded important activities that are within the scope of SynBio as currently understood and defined. For example, the SC considered applying parameters such as the degree of novelty of function or construction, as well as the number, size or complexity of synthetic or modified genetic elements. In each case, major SynBio activities occur at both extremes of the possible gradient of parameter values (e.g., very little vs. very high degree of functional novelty). In the remaining cases, the relevant aspects were so abstract that they are impossible to operationalise. For example, it is not meaningful to discriminate "how much rational design or engineering concepts had gone into designing a specific organism", as the outcome of the activity will be identical, independent of the psychological process or conceptualisation by the "engineer".

[28]As defined in Articles 2 (2) of the European Directives 2001/18/EC and 2009/41/EC and not necessarily encompassed by the techniques described in the corresponding Annexes of the Directives (see Annex V)

Since SynBio was considered an extension of genetic modification, the SC discussed the possibility of a sliding scale model that places activities on a continuum scale from classical GM to extreme SynBio. It was concluded that any attempt to reduce a complex multidimensional development such as SynBio to a single parameter (or small number of parameters) is artificial and arbitrary. However, most importantly, it would be impossible to apply for the identification and assessment of SynBio-related risks in the subsequent Opinions, because major scientific developments would not be covered and would have to be treated in an *ad hoc* manner.

A critical question in defining SynBio is deciding whether or not the definition needs to distinguish it from GM, biotechnology and other overlapping areas that already have definitions embodied in regulations.

The SC discussed discriminating SynBio from GM, because this was considered the core of the debate and the essence of the mandate questions. SynBio is currently under the existing risk assessment and regulatory frameworks for GM. However, it is not clear in which areas SynBio will go beyond the current GM framework and what the gaps are in the current risk assessment procedures. These will be discussed in detail in the second part of the Opinion in SynBio. After extensive debate, the conclusion was that attempting to identify a clear separation between GM and SynBio is currently not practical.

The following list of SynBio concepts and tools functions as an interpretative guideline to the operational definition, indicating recent technological and conceptual developments that underpin the acceleration and facilitation of GM that constitutes SynBio. This list is indicative rather than exhaustive and is expected to change over time as SynBio activities evolve. More information on the various tools and concepts on this list can be found in Section 3.

Concepts	Tools
Standardisation	BioCAD software
Modularisation	Robotic cloning
Hierarchical abstraction	Metabolic modelling
Decoupling of design and fabrication	Protein engineering
Orthogonality	DNA databases and registries
Refactoring	Part and device libraries
	Regulatory circuits
	DNA synthesis
	Gene and genome assembly
	Genome editing
	MAGE, CRISPR, TALENs, zinc fingers
	Microscopy
	Molecular profiling

Inclusion criteria: SynBio includes any activity that aims to modify the genetic material of living organisms as defined in CPB[29]. SynBio uses all available technologies for genetic modification, but in particular aims at the acceleration and facilitation of the process; this includes increasing its predictability. Therefore, for risk assessment, the SC broadly considers recent advances in tools, concepts and technologies that currently facilitate

[29]"Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology; (CPB, page 4, article 3, "Use of terms"). Living and genetically MOs terms are interchangeable for the purposes of this Opinion.

and accelerate the generation of GMOs, including those listed in the previous paragraph and in Section 3.3.1. The WG recognises that "advances" is a relative term: the degree of progress, acceleration and facilitation will always be in relation to a specific point in time. The SC will focus on identifying qualitative or quantitative changes in the type or scope of genetic modifications, which potentially create new risks or opportunities. This also includes consideration of non-viable, non-reproducing goods and materials generated by or through the use of such living GMOs.

Exclusion criteria: SynBio as defined here excludes work on biological entities that are not capable of replication or of transferring genetic material, according to the definition of a living organism in the CPB and in accordance with Article 2 (1) of the Directives 2009/41/EC and 2001/18/EC (see Annex V). Pre-life work, e.g. on protocells, is in the domain of chemistry as long as it does not produce living organisms. Protocells are important in the preparatory work in SynBio *research*, contributing to the long-term aims of SynBio.

Based on the current knowledge about scientific, technical and commercial developments and a comprehensive survey of the existing scientific definitions of SynBio, the following science-based operational definition of SynBio is proposed:

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.

This operational definition incorporates those common principles of existing definitions of SynBio that will potentially contribute to an operational definition using specific and preferably measureable inclusion and exclusion criteria, as described above. This operational definition reflects the working understanding of present and foreseeable technological advances.

The definition has the advantage that it does not exclude the application to SynBio of the relevant and large body of RA and safety guidelines developed over the past 40 years of GM work. Nor does it exclude extensions of that work, if needed, to account for recent technological advances as mentioned in Section 3.3.1.

The present definition also enables the rapidly advancing nature of GM technologies and adds an important nuance that supports the need for on-going updates of risk assessment methods, which will be addressed in Opinion II. The above definition includes genetic modification as presently defined, as well as current and expected future developments in SynBio.

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 the understanding 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 which 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.

The following table provides an overview of the considered criteria and the reasoning that lead to the conclusion that these criteria do not deliver clear cut-off points or thresholds that scientifically discriminate SynBio from other areas of GM.

Criteria discussed	Challenges
A considerable/substantial proportion of the resultant genetic material has been chemically synthesised	Although quantification of the amount or proportion of chemically synthesized genetic material is possible, any threshold would be arbitrary
The resultant genetic material or a part of it is newly designed	Although quantification of the amount of newly designed genetic material is possible, any threshold would be arbitrary
A significant proportion of the genetic material has been intentionally removed to develop a minimal functioning genome and/or a production chassis	Although quantification of the amount of removed material is possible, any threshold would be arbitrary
Standardised modular genetic parts have been utilised to rationally (re)design and assemble new or altered biological functions leading to new products; for example when a foreign pathway or genetic circuit has been introduced into a species in which it did not exist before	A limited number of standardised modular genetic parts have already been used in the past in genetic engineering. While SynBio tries to enhance this approach there is no agreed- upon parameter measuring the degree of standardisation.
A genetic construct that contains non-canonical heritable material	This criterion may be suited to discriminate xenobiology from other fields of SynBio, but does not help discriminating SynBio and GM.

With regard to risk assessment it is important to establish when it cannot be carried out following the framework established in Directives 2001/18/EC and 2009/41/EC and other relevant documents, e.g. EFSA guidance. It is important to realise that, rather than having one relevant reference point, GM, we have to consider four distinct reference points as illustrated in Figure 1.

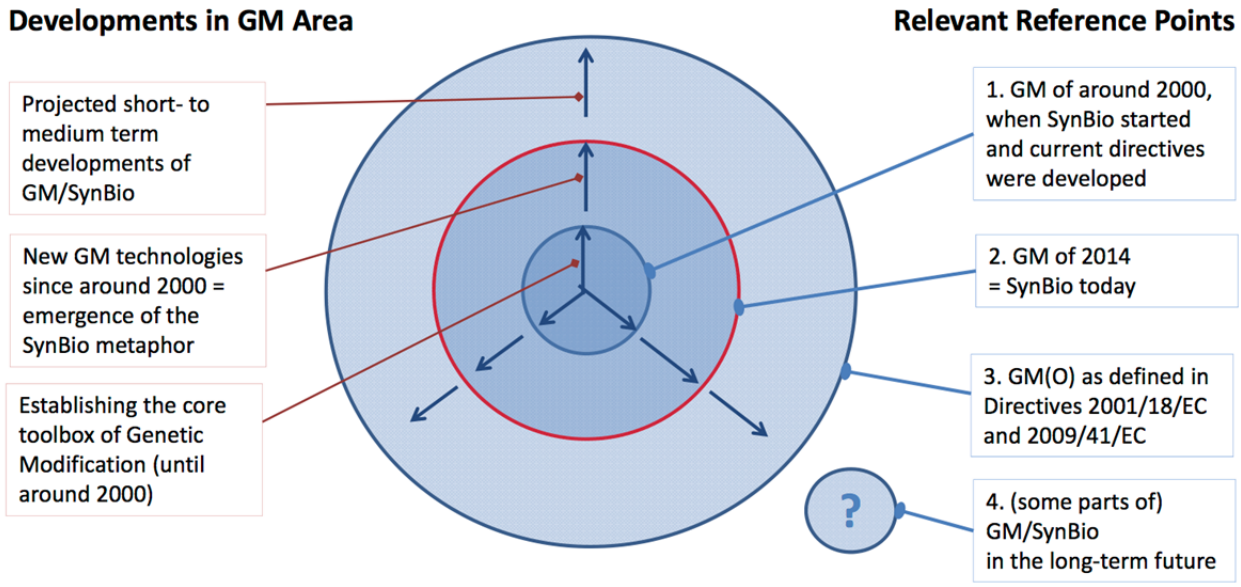

Figure 1. The relationship between SynBio and GM considering four different reference points: *1) The first reference point is GM as practised ca. 2000, when SynBio began to emerge and the current regulatory framework for GM was developed. 2) The second reference point is GM as practised in 2014, the current situation of GM, with developments beyond those in 2001. 3) The third reference is the official reference point requested by the Mandate of the working group: the definition of GMO provided in Article 2(1) of the Directives 2001/18/EC and 2009/41/EC, supplemented by the definition of LMO in the Cartagena Protocol on Biosafety. These definitions underlie current GM regulatory and legal frameworks in the EU and encompass a broad range of genetic modifications including those beyond what is practised today. 4) The fourth reference point takes into account the projected potential developments beyond the current state-of-the-art in GM and SynBio that will move beyond the scope of GM as it is defined in Article 2(1) of Directives 2001/18/EC and 2009/41/EC.*

4 OPINION

This Opinion is focused on answering the following questions from the EC on SynBio:

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

Over the past decade, a collection of technologies, methods and principles has emerged to progress towards the development of concepts and tools enabling for faster and easier design and manufacturing of GMOs. These approaches are important for responsibly addressing societal challenges such as health, energy and food security. These technologies, methods and principles are referred to as SynBio and include conceptual development in the area of genetic engineering with the adoption of classical engineering concepts like standardisation and modularisation, which scientists are attempting to apply to the engineering of biological systems. 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.

Existing definitions of SynBio mostly emphasise modularisation and related engineering concepts as the main drivers for accelerated and facilitated GMO design, manufacture and exploitation. However, for the risk assessment, an operational definition of SynBio needs to be provided, which is derived from the working understanding of SynBio as a collection of conceptual and technological advances that aims to enable faster and easier design and manufacturing of GMOs. These approaches are expected to make an important contribution towards responsibly addressing societal challenges in the areas of health, energy and food security (e.g. OECD, 2014).

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 term 'operational definition' is understood as a definition that makes it possible to unequivocally decide whether an activity is or is not SynBio based on present knowledge and understanding of the field. However, this definition may evolve as the understanding of 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 Biosafety, i.e. "any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids" and in Article 2(1) of the Directives 2009/41/EC and 2001/18/EC (Annex V). Of course, this does not exclude the consideration of non-viable, non-reproducing goods and materials generated by or through the use of such living GMOs.

GM involves the modification of living organisms with heritable material, independent of the chemical nature of the heritable material and the way in which it has been manufactured. SynBio uses all available technologies for genetic modification, but in particular aims at the acceleration and facilitation of the process, which includes increasing its 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 to reflect 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, these criteria do not deliver clear cut-off points or thresholds that scientifically discriminate SynBio from other areas of GM. Additional criteria including the complexity of the genetic modification, the speed by which modification was achieved, the number of independent modifications, or the degree of computational design methods used, alone or 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 definitions is provided in Annex III to this Opinion. Existing definitions are focused on conceptual advances within the scientific community. These conceptual definitions are not operational and not fundamental, as they are not based on quantifiable and currently measurable criteria. To address this deficiency in existing definitions and to enable our practical work on risk assessment, the SC suggests the science-based operational definition of SynBio in the response to question 1.

This operational definition incorporates those common principles of existing definitions of SynBio that will potentially contribute to an operational definition using specific and preferably measureable inclusion and exclusion criteria, as described above. This operational definition reflects our working understanding of present and foreseeable technological advances.

This definition has the advantage that it does not exclude the application to SynBio of the relevant and large body of RA and safety regulations developed over the past 40 years of GM work. Nor does it exclude extensions of that work, if needed, to account for recent technological advances such as standardised genetic parts combined with circuit libraries and engineering methods, protocells, minimal cells and designer chassis, xenobiology, large-scale DNA synthesis, and whole-genome editing.

The present definition also enables the rapidly advancing nature of GM technologies and adds an important nuance that supports the need for on-going updates of risk assessment methods, which will be addressed in Opinion II.

5 CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this opinion was open on the website of the EU scientific committees from 06 June 2014 to 21 July 2014. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders. In total 64 comments were received from 21 organisations or individuals.

Among the organisations participating in the consultation, there were universities, public health institutions, 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. In a significant number of cases, outlined in this document, this resulted in changes and corrections in the Opinion.

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_21_en.htm

6 MINORITY OPINION

None

7 ABBREVIATIONS AND GLOSSARY OF TERMS

- Biosafety level (BSL)
- Cartagena Protocol on Biosafety (CPB)
- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
- 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)
- International Genetically Engineered Machine (iGEM)
- Living Modified Organisms (LMOs)
- Multiplex Automated Genome Engineering (MAGE)
- Ministry of Science and Technology (MOST)
- Multiplex Automated Genome Engineering (MAGE)
- Nagoya Protocol (NP)
- National Institutes of health (NIH)
- 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)
- 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)
- Xeno Nucleic Acids (XNA)
- World Health Organisation (WHO)

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9 ANNEXES

9.1 Annex I

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 Synthetic Biology

Scope and definition of the phrase "Synthetic Biology"

1. What is Synthetic Biology 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 "Synthetic Biology"? 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 Synthetic Biology 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 Synthetic Biology research?

6. If existing methodologies are not appropriate to assess the potential risks associated with activities related to and products arising from Synthetic Biology 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 Synthetic Biology?

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

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 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 in question 3. 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.

9.2 Annex II: Summaries on FP projects and iGEM

SYNBIOLOGY: A European perspective on synthetic Biology. An Analysis of Synthetic Biology Research in Europe and North America (2003-2006, FP6-2003-NEST-B4 Project 015357): Objectives: 1) to provide a sector analysis to assist the EC in furthering its understanding of the SynBio sector, on the main actors in the sector, on the geographic distribution of this research and on what funding was available; 2) to disseminate the sector information and analysis to all interested stakeholders and the general public. Reports: Synthetic Biology Research – Literature & Statistical Review; SynBio Research Assessment; Europe/North America Comparative Assessment

BIOMODULARH2: Engineered Modular Bacterial Photoproduction of Hydrogen (FP6-NEST Pathfinder Synthetic Biology project num. 043340): Objectives: to design reusable, standardised molecular building blocks that would produce a photosynthetic bacterium containing engineered chemical pathways for competitive, clean and sustainable hydrogen production; to establish a systematic hierarchical engineering methodology (parts, devices and systems); to design artificial bacterial systems using a truly interdisciplinary approach that decouples design from fabrication; to construct biological molecular parts by engineering proteins with new enzymatic activities and molecular recognition patterns, by combining computational and *in vitro* evolution methodologies; and to create an anaerobic environment within the cell for an optimized, highly active iron-only hydrogenase by using an oxygen consuming device, which is connected to an oxygen sensing device and regulated by artificial circuits. Approach: The engineering approach was to provide the next generation of SynBio engineers with a toolbox to design complex circuits of high potential industrial applications such as the photo-production or photo-degradation of chemical compounds with a very high level of integration. The project targeted on a cyanobacterium, a very chemically rich and versatile organism highly suitable for modelling, to be used as the future platform for hydrogen production and biosolar applications. Results: Novel devices were designed (e.g. input/output, regulatory and metabolic) by combining parts and by using the emerging knowledge from systems biology. Circuits of devices were designed applying control engineering and optimisation.

Biotechnology TESSY: Towards a European Strategy in Synthetic Biology (2007-2008, Contract No. 043449): Objectives: to specifically map the current state of SynBio in Europe as the first step towards developing the field. The main tools at TESSY's disposal were a series of workshops, surveys and expert interviews. The results are summarized in the following documents: Working paper on results available from the other EU-funded projects; Working paper on databases for synthetic biological parts and other supportive infrastructures; Concepts to approach the roadmap goals; Results of the participatory approach; Final Roadmap towards SynBio in Europe; http://www.tessy-europe.eu/news.html

SynBioAssess (TESSY-result): SynBioAssess is a set of indicators that illustrate the fields, which have an impact on SynBio and/or are impacted by SynBio. The tool helps to collect all necessary data in SB and/or identify additional data requirements. It establishes a rational basis for decision-making on future funding and thus increases transparency in decision-making. The presentations given at the TESSY implementation workshop can be downloaded under http://www.tessy-europe.eu/news.html.

Biology SYNPLEXITY: Dynamics and complexity in synthetic protein networks (2007-2009, Marie Curie action): Objectives: to implement synthetic protein network motifs (feedback loops, toggle switches) operating in human cell lines; design of swappable interfaces that enable the exchange and rewiring of the different components; raw building blocks derived from modular proteins that transduct signals via auto-inhibition or spatial proximity between individual domains. Molecular engineering was assisted by computational protein design. Individual domains were labelled with genetically targeted small molecule fluorescence markers to follow and verify their status and interaction *in vivo*. This had yield parameters for the design and simulation of different networks, which could have been tested *in vivo*.

CELLCOMPUT: Synthetic protein networks (MOBILITY) (NEST, FP6, Biological computation built on cell communication systems): This project explored the concept of future robust biological computing. An additional application of such a system would be treating diseases in a targeted way. This concept involves specifically designed cells that would detect diseased tissues in the human body and produce the compounds necessary to treat the sickness. The result would be a higher concentration at the site of disease with fewer side effects in the rest of the body. Objectives: developing new approaches to cell communication systems that generate building blocks for biological computation devices. Biological computing addresses shortcomings that impact our lives including helping researchers devise more innovative methods for disease treatment. The project provided insight on how complex devices consisting of two, three or more programmed cells can be designed and constructed and form building blocks for such devices.

SYNBIOSAFE: Safety and Ethical Aspects of Synthetic Biology. Biological computation built on cell communication systems (2007-2008, FP6 NEST): Objectives: to stimulate a European debate on these issues at an early stage. Past experiences, especially in the field of GM-crops, have shown the importance of an early bio-safety and ethics debate. The community recognized this need, but discussions are fragmentary. SYNBIOSAFE started by interviewing experts in SynBio, bioethics and biosafety to gather facts and opinions on some of the following questions: How should research institutions and industry be regulated to prevent misuse and accidents without hindering development in SynBio? How should commercial DNA synthesis companies ensure that orders requested by their clients are not used to produce dangerous pathogens? What happens when a technology that can greatly benefit society also has military applications? How do we balance biosafety with academic freedom? Answering questions like these will help the project partners produce advice on risk assessment, safety, ethics, intellectual property rights and communication for researchers, stakeholders and the public. A second strand of the project focuses on ethics and public perceptions of SynBio. How will people respond to the potential of SynBio? Will they recognise its benefits? Are there ethical boundaries that the public will accept or impose, and will these boundaries change over time? The project intended to organise an e-forum for debates, media briefings, and an international workshop. SYNBIOSAFE aimed to help the EU develop its SynBio expertise in a responsible and socially acceptable manner. This is world-changing technology, and it is essential that foundations are in place to ensure it is used for the best. SYNBIOSAFE publications: Safety, Security and Ethical Aspects of Synthetic Biology.

TARPOL: Synthetic Biology for the Environment (CSA-CA): Targeting environmental pollution with engineered microbial systems a la carte (2008-2010, KBBE-2007-3-3-01): 'Targeting environmental pollution with engineered microbial systems a la carte' was an EU-funded initiative for directing and coordinating SynBio research in Europe. The project included a number of EU institutions to encourage more collaboration and interdisciplinary projects in the field. Objectives: to promote SynBio as a discipline both publicly and academically, and to develop material, technical and software resources. Project activities included several workshops and conferences, as well as research and database development at various institutions. TARPOL produced several useful molecular and software tools for synthetic biologists. Molecular tools included streamlined genetic systems and selection of useful strains of microorganisms. Software included a database of useful genes and genetic elements as well as computational tools for studying microbial physiology. By combining the genetic toolbox already available with engineering disciplines and computer sciences, TARPOL helped spur new approaches to environmental pollution problems. New SynBio tools could potentially be applied to, for example, carbon capture, storage and recycling, as well as to soil and water bioremediation.

BASYNTHEC: Synthetic biology for biotechnological applications (CP-FP): Bacterial Synthetic Minimal Genomes for Biotechnology (2010-2012, FP7, KBBE-2009-3-6-05): Objectives: to combine computational and experimental biology approaches with novel high-throughput methodologies to reduce and modify à la carte the chromosome of Bacillus subtilis, a genetically tractable bacterium and one of the key microbes used as a Cell Factory in biotechnology. Simpler B. subtilis strains with reduced energy consumption for self-maintenance have been designed and constructed by removing some potentially expensive cellular processes. The cells with the lowest experimentally determined waste of energy and with industrially relevant phenotypes will be engineered to reroute the flux devoted to biomass formation through rational modifications of the complex metabolic regulations and have been used as biotechnological platforms to plug in synthetic modules. For this purpose, BaSynthec developed a model-driven approach to design and engineer the strains with predetermined features, with a particular focus on unrestricted metabolic activity and the plug-in of synthetic functional modules. This strategy was based on the recent development of two complementary modelling approaches for B. subtilis: i) a genome-scale model of genetic and metabolic regulatory networks associated with a novel method called “Resource Balance Analysis” defining the formal background of model-based approaches for engineering strains; and ii) the development of a new genome-scale metabolic model of B. subtilis which is the most complete and accurate that exists today. Two pathways of high biotechnological relevance will be used for establishing the proof-of-principle of the assembly of functional synthetic modules: i) the vitamin B5 biosynthetic pathway, and ii) the secretion machinery for the export of extra-cellular enzymes. It was anticipated that validated simpler bacterial strains, together with the modelling framework generated by BaSynthec, would be used as generic biotechnological platforms to better control and exploit cell metabolism in industrial processes.

ST-FLOW: Towards standardisation in Synthetic Biology (CP-IP): Standardization and orthogonalization of the gene expression flow for robust engineering of NTN (new-to- nature) biological properties (2011-2014, FP7, KBBE.2011.3.6-03): This project merged the efforts of 15 leading European and US

research groups for developing material and computational standards that enabled the forward-design of prokaryotic systems with a degree of robustness and predictability that would not be possible with customary Genetic Engineering. The central issue at stake was the identification and implementation of rules that enables the conversion of given biological parts assembled with a set of principles for physical composition into perfectly predictable functional properties of the resulting devices, modules and entire systems. ST-FLOW focuses on each of the steps that go from assembling a DNA sequence encoding all necessary expression signals in a prokaryotic host (by default, E. coli) all the way to the making of the final product or to the behaviour of single cells and populations. Two complementary approaches will be adopted to solve the conundrum of physical composition vs. biological functionality of thereby engineered devices. In one case (bottom up), large combinatorial libraries of gene expression signals were to be merged with suitable reporter systems and the input/output functions examined and parameterized in a high-throughput fashion. The expected outcome of this effort was to establish experience-based but still reliable rules and criteria for the assembly of new devices and systems, following the same physical composition rules or adopting CAD design. Yet, many outliers (combinations that do not follow the rules) were expected, and making sense of them was the task of the complementary top-down approach. In this case, ST-FLOW was revisited and some gaps in our knowledge of the gene expression flow (transcription, mRNA fate, translation) need to be addressed for engineering functional devices from first principles. Ethical, legal and societal issues were also examined in a context of public dialogue and sound science communication.

METACODE: Applying Synthetic Biology principles towards the cell factory notion in biotechnology (CP-FP): Products from methanol by synthetic cell factories (PROMYSE) and Code-engineered new-to-nature microbial cell factories for novel and safety enhanced bio- production (201-2014, FP7, KBBE.2011.3.6-04): Objectives: to preform genetic code engineering in microbial strains with parallel recruitment of novel bio-orthogonal chemistries for mass production of desired protein/peptide based products. In combination with computational and classical chemical synthetic approaches as well as chemo-informatics, enzyme-guided evolution, synthetic metabolism, and directed evolution of microbial strains, artificial industrial microbial strains were planned to be designed enabling access to genetically robust and safe strains with added/novel functionalities and topologies from renewable resources. These strains will be characterized with an alternative reading of the genetic code (genetic firewall) and with predetermined chemistries (metathesis), as well as necessary robustness for efficient industrial use. The plan was to demonstrate the power of orthogonalization as a biosystems engineering strategy and solve industrially relevant bio-production problems, such as peptide and protein production beyond the canonical set of the 20 proteinogenic amino acids. The plan was also to expand the arsenal of biologically available chemical reactions. While the first objective was expected to have a strong impact on pharmaceutical applications, the latter was essential to the transition of a chemical to a biochemical industry at the heart of the Knowledge-Based BioEconomy.

ERASynBio: Synthetic biology – ERA-NET. Development and Coordination of Synthetic Biology in the European Research Area (2011-2013, FP7, KBBE.2011.3.6-06): Objectives: to promote the robust development of SynBio by structuring and coordinating national efforts and investment. They planned to develop a white paper to support the emergence of national SynBio programmes and lay the ground for transnational funding activities via joint calls in the project. The plan was to

stimulate and tackle the interdisciplinary nature and immaturity by offering training and educational possibilities, establishing an interdisciplinary advisory board and inviting observers of other funding organisations. It was to provide extensive dialogue options and exchanges for the scientific community. Close collaboration between academia and industry aimed to fertilize the innovation process. To adhere to ethical, legal and societal aspects as well as to technical issues like standardization and infrastructure development, they planned to trace and integrate the ongoing work and research on these framework conditions and integrate them in the white paper. The aim was to create the ERA of SynBio in parallel with the development of the scientific community.

SYBHEL: Ethics and new and emerging fields of science and technology (2009-2012, FP7, SiS-2008-1.1.2.1): Objectives: to investigate the ethical, legal and policy issues that raised by SynBio in respect to human health and wellbeing. It was the first study to focus specifically on ethical, legal and policy the implications of SynBio in respect to human health and wellbeing. SYBHEL was a three-year EU-funded project. The main objectives: 1. Carry out high quality ethical research and evaluation of how SynBio will impact human health and well-being. 2. Underpin research with a consistent awareness of SYBHEL crosscutting themes, namely: the definition of SynBio; scientific research (including documenting and regularly updating the state-of-the-art); safety and justice. 3. Create a hub for researchers and policymakers interested in ethical, legal and social issues arising in SynBio as it applies to human health to meet and exchange ideas. 4. Debate and agreed recommendations for regulation and commercialisation of SynBio as it pertains to human health and well-being. 5. Determine a strategy for policy deliberation for SynBio and human health. Main results: Final report containing recommendations to the EC and regulatory agencies concerning government regulation and recommendations to the EC, and to European and national research policy and funding organisations, concerning anticipatory governance.

SYNTH-ETHICS: Ethical and regulatory challenges raised by synthetic biology (FP7): Objectives: to address the ethical, legal and social implications of the emerging field of SynBio with a special focus on biosafety and biosecurity and on notions of life. The project aimed to contribute to the common understanding of SynBio and the ethical, legal and social issues involved in EU member states and to the shaping of a distinct European approach without ignoring the discussions and developments in the US and elsewhere. The overall aim of the project was to contribute substantially to the development of a European approach to SynBio. The specific aims of the project were: 1. to identify actual and emerging ethical issues raised by developments in SynBio and the embedding of the developed technologies in society; 2. to trace and analyse the public discourse on these issues; 3. to analyse whether these ethical issues, and the concerns raised in the public discourse, can be adequately dealt with the current normative frameworks existing in SynBio and in closely related fields such as nano- and biotechnology genetic engineering, and identify shortcomings; and 4. to analyse topics in SynBio on which EU policy and regulation might be required and to make recommendations on these topics. This document is the report on ethical issues and public discourse. It provides for an overview of ethically sensitive issues in SynBio in the form of a state-of-the-art report; a combined overview and summary of information obtained through interviews, a survey, a group decision room-session and an expert workshop; an in-depth analysis of outstanding philosophical issues; an overview of the public discourse on SynBio. Synthethics: Ethical and regulatory challenges raised by SynBio; This report gives an overview of the most important findings of SynthEthics

second work package, “Ethical and regulatory challenges raised by SynBio”; Elaborate overview of all relevant areas in law that were identified for SynBio. Regulatory areas included: issues connected to GMOs, SynBio and biofuels, SynBio and biomedical applications, applications of SynBio in cosmetics, issues of intellectual property, bio-Informatics, SynBio and occupational health, issues connected to human rights, issues connected to precaution, the role of soft law and the convergence of ethical and legal principles into (mainly soft) regulatory tools. Includes a survey of scientists to gain insight in the knowledge, views and opinions of expert scientists working in SynBio and related fields. All questions raised in this context were further elaborated through interviews with experts in policy making on regulating SynBio.

SYNENERGENE: Mobilisation and Mutual Learning Action Plans; - Synthetic biology – Engaging with New and Emerging Science and Technology in Responsible Governance of the Science and Society Relationship (2013-2017, FP7, SiS.2012.1.2-1): Objectives: to initiate various activities with a view to stimulating and fostering debate on the opportunities and risks of SynBio. Conceptualized as a so-called "Mobilisation and Mutual Learning Action Plan" (MMLAP), the project involved various academic and societal actors from Europe and other countries in numerous activities such as citizen consultations, theatrical debates, and monitoring activities. The stakeholders involved proposed to shape this new field together, engage in mutual learning and develop sustainable agendas for the future development of SynBio. Specific objectives: to make existing practices of RRI (Responsible Research and Innovation) in SynBio socially more robust, to mobilise new stakeholders to participate in discourse on SynBio, to involve the general public and specific “publics” and improve the quality of public participation by a wide variety of means, to analyse and to make available the results of all public dialogue and stakeholder-oriented activities to policy makers, other stakeholders and the public, to promote mutual learning processes between a wide variety of established and new stakeholders in discourse on SynBio, stimulating reflection and activities on novel and innovative avenues to an inclusive governance framework in accordance with a European concept of RRI and of high international visibility, and to help developing sustainable agendas for RRI in SynBio which systematically take into account the views of citizens involved in public communication activities.

iGEM: By ‘making biology easier to engineer’ SB also facilitates ‘the contribution to scientific innovation from people who are not considered as professional experts in the traditional sense’ (Zhang, 2013). This is best exemplified by the annual international Genetically Engineered Machine (iGEM) competition, in which high school, undergraduate, and graduate student teams design and implement biological systems to address global issues such as biofuel production and disease containment. The popularity of the competition has spread quickly since its 2003 inauguration at the Massachusetts Institute of Technology (Smolke, 2009), and in 2013 over 200 teams participated from all around the world. From the beginning, iGEM has been a showcase and test bed for some of the most innovative applications of synthetic biology.

Exemplary projects from the 2013 edition of the iGEM competition worked on the development of a biosensor for arsenic contamination in drinking water (Team Buenos Aires), probiotics for bees to prevent colony collapses caused by pathogenic fungi (Team National Yang Ming University, Taipei), and novel ways to fight tuberculosis infections (Team Paris Bettencourt).

iGEM is contributing to the emergence of a generation of self-identified synthetic biologists, the first of whom are reaching tenure-track and industry leadership positions. Dafni and Delebecque (Glinos and Delebecque, 2014) (to be published) analysed the past 10 years of the competition, and the interactive map of the iGEM ecosystem they developed is already available online[30].

Their main conclusions were:

1. The sharing philosophy behind the iGEM competition has significantly promoted the open access culture and standardization of biological parts, and has challenged traditional intellectual property regimes.

2. The reward structure of the competition has been efficient in fostering scientific breakthroughs and has encouraged the reuse and continued iterative improvement of standardized biological parts.

3. Finally, iGEM has been encouraging responsible scientific governance by having the teams investigate human impacts of synthetic biology.

[30]http://igem.org/Previous_iGEM_Competitions

9.3 Annex III: Synthetic biology - definitions

Source	Definition	Key words/ focus
European Commission Report of a NEST High-Level Expert Group: "Synthetic Biology Applying Engineering to Biology" 2005	Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature. This engineering perspective may be applied at all levels of the hierarchy of biological structures from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of biological systems in a rational and systematic way.	Engineering Principles applied to biology; Rational design and synthesis of complex (novel) biological systems.
Synthetic Biology project EU FP6[31] 2006	Synthetic biology is the engineering of biological components and systems that do not exist in nature and the re-engineering of existing biological elements; it is determined on the intentional design of artificial biological systems, rather than on the understanding of natural biology.	(Re) engineering of novel biological components and systems through intentional design.
Synthetic Biology 3.0[32] 2007	Synthetic biology is a new and rapidly emerging discipline that aims at the (re-)design and construction of (new) biological systems.	(Re-) designing and synthesis of (new) biological systems.
Synthetic Biology 4.0[33] *2008*	Synthetic Biology is a new approach to engineering biology, with an emphasis on technologies to write DNA. Recent advances make the de novo chemical synthesis of long DNA polymers routine and precise. Foundational work, including the standardization of DNA-encoded parts and devices, enables them to be combined to create programs to control cells. With the development of this technology, there is a concurrent effort to address legal, social and ethical issues.	Engineering biology; DNA coded parts and devices; Control of cell function.
UK parliamentary office for Science and Technology Post Note[34] 2008	Synthetic biology aims to design and build new biological parts and systems or to modify existing ones to carry out novel tasks.	New or modified biological parts and systems for novel tasks.
Towards a European Strategy for Synthetic Biology - EU FP6[35]	Synthetic Biology aims at designing biological systems that do not exist in nature using engineering principles or re-designing existing ones to better understand life processes, to generate and assemble functional modular components, and to develop novel applications or processes.	(Re)design of (novel) biological systems; Functional modular components for novel applications and processes.
Ethic report [36]	A definition of synthetic biology should therefore include: 1.The design of minimal cells/organisms (including minimal genomes); 2. The identification and use of biological 'parts' (toolkit); 3. The construction of totally or	Identification, design and use of (artificial) biological parts.

[31] http://www2.spi.pt/synbiology/documents/news/D11%20-%20Final%20Report.pdf (accessed 24 06 2013)
[32] http://www.syntheticbiology3.ethz.ch/index.htm (accessed 24 06 2013)
[33] http://sb4.biobricks.org/field/ *(accessed 24 06 2013)*
[34] http://www.parliament.uk/documents/post/postpn298.pdf (accessed 24 06 2013)
[35] http://www.tessy-europe.eu/public_docs/TESSY-Final-Report_D5-3.pdf
[36] http://ec.europa.eu/bepa/european-group-ethics/docs/opinion25_en.pdf (accessed 03 07 2013)

Source	Definition	Key words / focus
	partially artificial biological systems.	
Synthetic Biology Org[37]	Synthetic Biology is (a) the design and construction of new biological parts, devices, and systems, and (b) the redesign of existing, natural biological systems for useful purposes.	Design of new biological parts, devices and systems; Redesign of existing, natural biological systems.
Richard Kitney for "Synthetic Biology From Science to Governance: A workshop organised by the European Commission's Directorate-General for Health & Consumers"[38]. 2010	Two complementary definitions for SynBio: (a) designing and making biological parts and systems that do not exist in the natural world using engineering principles, and (b) redesigning existing biological systems, again using engineering principles.	Designing new or redesigning the existing biological systems through engineering processes
Presidential Commission for the Study of Bioethical Issues, Report on Synthetic Biology[39] 2011	Synthetic biology is the name given to an emerging field of research that combines elements of biology, engineering, genetics, chemistry, and computer science. The diverse but related endeavors that fall under its umbrella rely on chemically synthesised DNA, along with standardised and automatable processes, to create new biochemical systems or organisms with novel or enhanced characteristics.	Combines different scientific disciplines; uses synthetic DNA to develop new biochemical systems or organisms with novel or enhanced characteristics.
A synthetic biology roadmap for the UK[40] 2012	Synthetic biology is the design and engineering of biologically based parts, novel devices and systems as well as the redesign of existing, natural biological systems.	(Re)design/engineer-ing of biologically based parts, novel devices and systems; Engineering of biologically based parts, novel devices and systems Redesign of existing, natural biological systems
UNICRI[41] 2012	Synthetic Biology is the deliberate design of biological systems and living organisms using engineering principles	Design / engineering of biological systems and organisms.
Blake and Isaacs (2004)[42]	Synthetic biology is advancing rapidly as biologists, physicists and engineers are combining their efforts to understand and program cell function. By characterizing isolated genetic components or modules, experimentalists have paved the way for more quantitative analyses of genetic networks	Genetic components and module
De Vriend (2006)[43]	Synthetic biology is a newly emerging scientific	Convergence of various

[37]http://syntheticbiology.org/ (accessed 24 06 2013)
[38]http://ec.europa.eu/health/dialogue_collaboration/docs/synbio_workshop_report_en.pdf (accessed 24 06 2013)
[39]http://bioethics.gov/sites/default/files/PCSBI-Synthetic-Biology-Report-12.16.10_0.pdf (accessed 24 06 2013)
[40]http://www.rcuk.ac.uk/documents/publications/SyntheticBiologyRoadmap.pdf (accessed 24 06 2013)
[41]http://www.unicri.it/in_focus/files/UNICRI%202012%20Security%20Implications%20of%20Synthetic%20Biology%20and%20Nanobiotechnology%20Final%20Public-1.pdf (accessed 03 07 2013)
[42]W. J. Blake, F. J. Isaacs, Synthetic biology evolves. Trends Biotechnol 22, 321 (Jul, 2004)

Source	Definition	Key words / focus
	field where ICT, biotechnology and nanotechnology meet and strengthen each other. Synthetic biology is a new trend in science and technology and a clear example of converging technologies	technologies.
Heinemann and Panke (2006)[44]	Synthetic biology is interpreted as the engineering-driven building of increasingly complex biological entities for novel applications.	Engineering driven complex biological entities for novel applications.
sc | nat, "Synthetic Biology" (2006)	Synthetic biology is a new research field, combining elements of gene technology and nanotechnologies with elements of the engineering sciences	Convergence of various technologies.
Drubin et. al. (2007)[45]	Synthetic biology refers to a variety of experimental approaches that either seek to modify or mimic biological systems	Approaches to modify or mimic biological systems.
ETC, "Extreme Genetic Engineering An Introduction to Synthetic Biology" (2007)	Synthetic Biology (also known as Synbio, Synthetic Genomics, Constructive Biology or Systems Biology) – the design and construction of new biological parts, devices and systems that do not exist in the natural world and also the redesign of existing biological systems to perform specific tasks.	(Re)design and construction of (novel) biological parts, devices, and systems to perform specific tasks.
ETC, "Extreme Genetic Engineering An Introduction to Synthetic Biology" (2007)	Synthetic biology is an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems	(Re)design and construction of (novel) biological pathways, organisms or devices,
Entus et al. (2007)[46]	Synthetic biology is a useful tool to investigate the dynamics of small biological networks and to assess our capacity to predict their behavior from computational models	A means to investigate and model biological networks.
IRGC[47], "Synthetic biology: risk and opportunities of an emerging field" (2008)	Most definitions of synthetic biology have two parts: synthetic biology is defined as the construction of completely novel biological entities, and the re-design of already existing ones	(Re)design of (novel) biological entities.
HSE, "Synthetic biology A review of the technology, and current and future needs from the regulatory framework in Great Britain" (2012).	Synthetic biology is a term used to cover areas of biochemistry research that is involved in the chemical synthesis of DNA, utilising biological agents or their components for potential application across a wide range of industrial sectors	Manipulation of synthetic DNA in biological systems.
The Royal Academy of Engineering "Synthetic	Synthetic biology aims to design and engineer biologically based parts, novel devices and	(Re)design/engineer novel systems and

[43]H. De Vriend, "Constructing Life. Early social reflections on the emerging field of synthetic biology" (2006)
[44]M. Heinemann, S. Panke, Synthetic biology-putting engineering into biology. Bioinformatics 22, 2790 (2006)
[45]D. A. Drubin, J. C. Way, P. A. Silver, Designing biological systems. Genes Dev 21, 242 (Feb 1, 2007).
[46]R. Entus, B. Aufderheide, H. M. Sauro, Design and implementation of three incoherent feed-forward motif based biological concentration sensors. Syst Synth Biol 1, 119 (Aug, 2007)
[47]IRGC, Risk governance of synthetic biology (revised concept note), 2009. IRGC, Guidelines for the Appropriate Risk Governance of Synthetic Biology (Policy Brief), 2010 http://www.irgc.org/issues/synthetic-biology/ ISBN 978-2-9700672-6-9

Source	Definition	Key words / focus
Biology: scope applications and implications" (2009[48]).	systems as well as redesigning existing, natural biological systems. Synthetic biology strives to make the engineering of biology easier and more predictable.	devices
A. Danchin, 'Synthetic biology: discovering new worlds and new words', EMBO reports; doi:10.1038/embor.2008.159 (2008)	The fundamental idea behind synthetic biology is that any biological system can be regarded as a combination of individual functional elements — not unlike those found in man-made devices. These can therefore be described as a limited number of parts that can be combined in novel configurations to modify existing properties or to create new ones.	Novel combinations of biological functional parts
EU Project 'Towards a European Strategy for Synthetic Biology' (TESSY, 2007-2008): www.tessy-europe.eu/	Synthetic biology uses nucleic acid elements or complex systems that are predefined and chemically synthesised in the laboratory by a modular approach. This approach aims to: 1. engineer and study biological systems that do not exist as such in nature, and 2. use this approach for i) achieving better understanding of life processes, ii) generating and assembling functional modular components, iii) developing novel applications or processes.	Synthetic, artificial, assembly of functional modular components, novel processes/ applications
Benner SA and Sismour AM, Synthetic Biology Nat Rev Genet 6:533-43 (2005)	[Synthetic biology] attempts to recreate in unnatural chemical systems the emergent properties of living systems … [the] engineering community has given further meaning to the title…to extract from living systems interchangeable parts that might be tested, validated as construction units, and reassembled to create devices that might (or might not) have analogues in living systems.	Artificial assembly of biological parts
Hastings Center, USA	To advance knowledge and create products that can promote human welfare, synthetic biologists seek to create biological systems that do not occur naturally as well as reengineer biological systems that do occur naturally.	Artificial biological systems through (re)engineering
UK Parliamentary Office of Science and Technology, POSTNOTE Number 298, January 2008	[Synthetic biology] describes research that combines biology with the principles of engineering to design and build standardised, interchangeable biological DNA building-blocks. These have specific functions and can be joined to create engineered biological parts, systems and, potentially, organisms. It may also involve modifying naturally occurring genomes to make new systems or by using them in new contexts.	DNA building blocks to engineer biological parts
Erasynbio's definition https://www.erasynbio.eu	Synthetic Biology is the engineering of biology: the deliberate (re)design and construction of novel biological and biologically based parts, devices and systems to perform new functions for useful purposes, that draws on principles elucidated from biology and engineering.	
The Netherlands Commission on Genetic	Description: Synthetic biology is seen as a technology that offers new possibilities for	Re-designing and synthesis of (new)

[48]Royal Academy of Engineering (2009) Synthetic Biology: scope, applications and implications. https://www.raeng.org.uk/societygov/policy/current_issues/synthetic_biology/default.htm. ISBN: 1-903496-44-6

Source	Definition	Key words / focus
Modification, 2013	biotechnological applications and research. It seeks to modify existing organisms and to design and synthesise new organisms.	biological systems.
The German Academy of Sciences Leopoldina, together with the German Academy of Science and Engineering and the German Research Foundation (DFG, 2009)	Description: Synthetic biology combines a wide spectrum of scientific disciplines and follows the principles of engineering science. Its chief characteristic is the modification of biological systems, which may also be combined with chemically synthesised components to produce new entities	Modification of biological systems / chemically synthesised components/ new entities
The Royal Netherlands Academy of Arts and Sciences, together with the Health Council of the Netherlands and the Advisory Council on Health Research[49]	Adopts definition of the European Commission Report of a NEST High-Level Expert Group: "Synthetic Biology Applying Engineering to Biology"): SynBio is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature. This engineering perspective may be added at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way	Rational design and synthesis of complex (novel) biological systems.
The Swiss Academy of Technical Sciences	Refers to definition of EASAC, (2011): Synthetic Biology: an introduction Synthetic biology is the application of engineering principles to biology. This may involve redesigning a living system so that it does something – manufacture a particular substance, perhaps – that it would not naturally do. Still more ambitious are attempts not merely to re-engineer living systems, but to fashion entirely new ones: to create life itself from non-living materials.	Engineering Principles applied to biology; (re) design and synthesis of complex (novel) biological systems.
Zentrale Kommission für die Biologische Sicherheit (2012) Monitoring der Synthetischen Biologie in Deutschland. http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/01_Allgemeine_Stellungnahmen_deutsch/01_allgemeine_Themen/Synthetische_Biologie.pdf?_blob=publicationFile&v=3	Ziel der Synthetischen Biologie ist es, biologische Einheiten wie z.B. Enzyme, genetische Schaltkreise oder Zellen so zu gestalten, wie sie nicht in der Natur vorkommen.	
Arjun Bhutkar, Synthetic Biology: Navigating the Challenges Ahead. J. BIOLAW &BUS., Vol. 8, No.2, 2005**.**	Rather than splicing in a gene from one organism to another, or forcing a mutation in a genome for a specific purpose, synthetic biology mainly concerns designing and building artificial regulatory elements into genomes or constructing a complete genome	

[49]Royal Academy of Engineering (2009) Synthetic Biology: scope, applications and implications. https://www.raeng.org.uk/societygov/policy/current_issues/synthetic_biology/default.htm. ISBN: 1-903496-44-6

Source	Definition	Key words / focus
	out of nucleotides"	

9.4 ANNEX IV: Regulatory framework that would apply to the various synthetic biology applications

GMO regulations

Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities L106: 1-38.

Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified microorganisms. OJ L 125, 21.05.2009, p. 75-97.

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1-23.

Regulation (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms. OJ L 287, 5.11.2003, p. 1-10.

Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. OJ L 268, 18.10.2003, p. 24-28.

Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006.

GMO medicinal products

Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency of the European Parliament. OJ L 136, 30.4.2004, p. 1-33.

Biological risks

Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community. OJ L 378, 31.12.1982, p. 58–62.

Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1.

Council Regulation (EC) No 1334/2000 of 22 June 2000 setting up a Community regime for the control of exports of dual-use items and technology. OJ L 159, 30.6.2000, p. 1.

Reg. 851/2004 establishing ECDC (disease outbreaks/communicable diseases control) The new decision 1082/2013 on serious cross-border threats to health.

Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Occupational health

Directive 2000/54/EC of the European Parliament and the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

New medicinal products

Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency of the European Parliament. OJ L 136, 30.4.2004, p. 1-33.

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 26.11.2001, p. 1-38.

Commission Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use. OJ L 159, 27.06.2003, p. 46-94.

Commission Directive 2003/94/EC of 8 October 2003 laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. OJ L 262, 14.10.2003, p. 22-26.

Medical Devices

Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. OJ L 169, 12.07.1993, p. 1-43.

Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices. OJ L 189, 12.07.1990, p. 17-36.

Council Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices. OJ L331, 7.12.1998.

Gene therapy, cell therapy and tissue engineering

Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 on genetically modified food and feed. OJ L 324, 10.12.2007, p. 121-137.

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 26.11.2001, p. 1-38.

Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. OJ L 102, 07.04.2004, p 48-58.

Directive 2002/98/EC of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 33, 08.02.2003, p. 30-40.

Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency of the European Parliament. OJ L 136, 30.4.2004, p. 1-33.

Clinical trials

Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. OJ L 121, 01.05.2000, p. 34-44 (amended in 2003 and 2005).

Cosmetic products

Directive 2002/98/EC of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 33, 08.02.2003, p. 30-40.

Council Directive 1976/768/EC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. OJ L 262, 27.9.1976, p. 169.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59-209.

Chemicals

REACH, the European Community Regulation on chemicals and their safe use (EC 1907/2006). It deals with the Registration, Evaluation, Authorization and Restriction of Chemical substances. The law entered into force on June 1, 2007.

Products intended for food and feed uses

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1 24.

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients**.** OJ L 043, 14.02.199, p. 1 – 6.

Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1 65.

No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.05.2008, p. 1–65.

Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7–15.

Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJL 354, 31.12.2008, p. 16–33.

Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91.

Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

9.5 Annex V: GMO Definition according to Directives 2001/18/EC and 2009/41/EC

Genetically Modified Organism (GMO) and Genetically Modified Micro-organism (GMM) are defined in Article 2 of the European Directives 2001/18/EC and 2009/41/EC respectively as follows: *'Genetically modified (micro-)organism shall mean a (micro-) organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'*. The wording '*altered in a way*' indicates that the focus is also on the process or the technique used to construct GMOs. Since the trigger for regulatory oversight of GMOs and GMMs is process-based, the Directives include annexes that provide additional information regarding the techniques:

i) that result in genetic modification (non-exhaustive list) (Annex I, Part A of Directive 2009/41/EC and Annex I A Part I of Directive 2001/18/EC, see Table 1 below)

ii) that are not considered to result in genetic modification (Annex I, Part B of Directive 2009/41/EC and Annex IA Part 2 of Directive 2001/18/EC, see Table 1 below)

iii) that result in genetic modification but yield organisms that are excluded from the scope of the Directives (Annex II Part A of Directive 2009/41/EC and Annex IB of Directive 2001/18/EC, see Table 1 below).

Thus, according to these Directives, a novel organism will fall under the scope of the GMO Regulation, if it has been developed with the use of certain techniques.

Table. The definition of a GMO according to EU Directives and its annexes

Directive 2009/41/EC	Directive 2001/18/EC
Article 2 (a) "micro-organism" shall mean any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture; (b) "genetically modified micro-organism" (GMM) shall mean a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Within the terms of this definition: (i) genetic modification occurs at least through the use of the techniques listed in Annex I, Part A; (ii) the techniques listed in Annex I, Part B, are not considered to result in genetic modification;	*Article 2* (1) "organism" means any biological entity capable of replication or of transferring genetic material; (2) "genetically modified organism (GMO)" means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination; Within the terms of this definition: (a) genetic modification occurs at least through the use of the techniques listed in Annex I A, Part 1; (b) the techniques listed in Annex I A, Part 2, are not considered to result in genetic modification. *Article 3.1* This Directive shall not apply to organisms obtained through the techniques of genetic

Directive 2009/41/EC	Directive 2001/18/EC
Article 3 this Directive shall not apply: - where genetic modification is obtained through the use of the techniques/methods listed in Annex II, Part A	modification listed in Annex I B.
Annex I Part A Techniques of genetic modification referred to in Article 2(b)(i) are, *inter alia*: 1. Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation. 2. Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism including micro-injection, macro-injection and micro-encapsulation. 3. Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.	*Annex I A* *Techniques referred to in Article 2(2)* *Part 1* Techniques of genetic modification referred to in Article 2(2)(a) are *inter alia*: (1) Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation; (2) Techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation; (3) Cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.
Annex I *Part B* Techniques referred to in Article 2(b)(ii) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs made by techniques/ methods other than	*Annex IA* *Techniques referred to in Article 2(2)* *Part 2* Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant

Directive 2009/41/EC	Directive 2001/18/EC
techniques/methods excluded by Annex II, Part A: (1) *in vitro* fertilisation; (2) natural processes such as: conjugation, transduction, transformation; (3) polyploidy induction.	nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex IB: (1) *in vitro* fertilisation, (2) natural processes such as: conjugation, transduction, transformation (3) polyploidy induction.
Annex II Part A Techniques or methods of genetic modification yielding micro-organisms to be excluded from the Directive on the condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs other than those produced by one or more of the techniques/methods listed below: (1) Mutagenesis. (2) Cell fusion (including protoplast fusion) of prokaryotic species that exchange genetic material by known physiological processes. (3) Cell fusion (including protoplast fusion) of cells of any eukaryotic species, including production of **hybridomas** and plant cell fusions. (4) Self-cloning consisting in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent) with or without prior enzymatic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes where the resulting microorganism is unlikely to cause disease to humans,	Annex I B Techniques referred to in Article 3 Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are: (1) Mutagenesis. (2) Cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

Directive 2009/41/EC	Directive 2001/18/EC
animals or plants. Self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular microorganisms.	