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 II
Risk assessment methodologies and safety aspects

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The Scientific Committees adopted this Opinion:

The SCENIHR at their plenary on 29 April 2015, the SCHER and the SCCS by written procedure on 4 May 2015

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to 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 risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases.

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

ABSTRACT

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

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

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

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

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

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

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

The framework for risk assessment of new SynBio developments may be addressed using current methodology used for GMO risk assessment. However, there are specific cases in which new approaches may be necessary. These include risks pertaining to 1)

routes of exposure and adverse effects arising from the integration of protocells into living organisms and future developments of autonomous protocells, 2) new xenobiological variants and their risk on human health and the environment that should be engineered for improved biocontainment, 3) DNA synthesis and direct genome editing of zygotes which enables modifications in higher animals within a single generation, and 4) new multiplexed genetic modifications which increase the number of genetic modifications introduced in parallel by large-scale DNA synthesis and/or highly-parallel genome editing and will increase the genetic distance between the resulting organism and any natural or previously modified organism.

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

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

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

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

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

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

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

A blue print of a general strategy for designing inherently safe applications of SynBio is demanding, because of the stochastic and probabilistic character of the underlying biochemical SynBio processes. General biocontainment approaches are based on 1)

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

Keywords: Synthetic biology; biotechnology; bioengineering; genetic engineering; microbiology; molecular biology; Regulatory framework; genetically modified organisms (GMO); risk assessment; risk assessment methodology; risk mitigation; Genetic part libraries; Minimal cells and designer chassis; Protocells and artificial cells; Xenobiology; DNA synthesis and genome editing; Citizen science; Do-It-Yourself biology.

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1 BACKGROUND

This Opinion is the second in a series of three on Synthetic Biology (SynBio) responding to questions from the European Commission (EC). The overall, legal and scientific background underlying these questions from the Commission were discussed in the first Opinion and research priorities will be addressed in the third Opinion.

1.1 General introduction

SynBio aims to design new biological systems that do not yet exist in nature. Synthetic biologists use engineering principles and re-design existing systems to better understand life processes. In addition, the objective is to generate and assemble functional modular components for the development of novel applications and processes such as synthetic life, cells or genomes. SynBio processes offer novel opportunities for the creation of new industries with profound economic implications for the European Union (EU) and other major economies. Just as advances in synthetic chemistry had a major impact on the shaping of modern societal and economic structures in the 19th and 20th centuries, SynBio promises substantial benefits for health, the environment, resource management and the economy. In addition to the 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 genetically modified organisms (GMOs) as defined under Directive 2001/18/EC on the deliberate release of GMOs and Directive 2009/41/EC on contained use of GM microorganisms (GMMs). Although most of the techniques analysed by the NT Working Group were focused on the direct implications on plant breeding, synthetic genomics, as a field within SynBio that may include techniques of genetic modification, was also considered. The Report from this Working Group was finalised in January 2012 (NTWG, 2012 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 was uncertain whether Directives 2009/41/EC, 2001/18/EC, and Section Annex V from the European GMO regulatory framework were the appropriate legislation to cover synthetic genomics and SynBio. The SynBio WG was established with the mandate to address these uncertainties and to explore the implications of SynBio, including but not limited to synthetic genomics and related technologies".

2 TERMS OF REFERENCE

The 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 2 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 1, 2014, the closing date for data considered for this Opinion, were identified and critically examined. 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 were included and commented upon in the Opinion. In some areas where the literature is particularly scarce, an explanation is provided for clarification. Detailed criteria for selecting studies were published in the SCENIHR Memorandum "Use of the scientific literature for human health risk assessment purposes, weighing of evidence and expression of uncertainty" (SCENIHR, 2012).

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

These questions are part of a set of 11 questions from the European Commission on SynBio (see Annex I). 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. Questions 4 through 8 are addressed in this Opinion II:

4. *What are the implications for human and 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?*[2]

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?*

[1]European Commission (2013) Request for a joint scientific opinion on Synthetic Biology. Brussels.
[2]http://eur-lex.europa.eu/resource.html?uri=cellar:303dd4fa-07a8-4d20-86a8-0baaf0518d22.0004.02/DOC_1&format=PDF and http://eur-lex.europa.eu/resource.html?uri=cellar:303dd4fa-07a8-4d20-86a8-0baaf0518d22.0004.02/DOC_2&format=PDF

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.[3]

In the first companion Opinion (SCHER, SCENIHR, SCCS, 2014), the first 3 questions on scope and definition were answered. The abstract from Opinion I is included in Annex II.

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

3 SCIENTIFIC RATIONALE

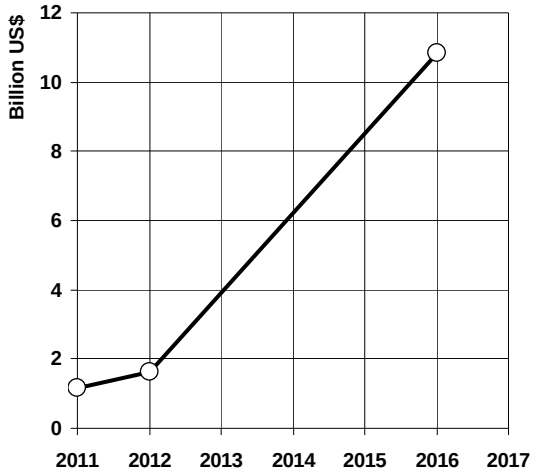
3.1 Introduction

3.1.1 Origin, achievements and impacts of SynBio

From 1904 through the 1930s, researchers at Carnegie Institution's Station for Experimental Evolution at Cold Spring Harbor pursued the formation of "synthetic new species" through the use of the novel technology of mutation-enhanced breeding to control evolution (Campos, 2010). The pioneers of molecular biology and genetic engineering in the 1970s and 1980s harnessed their ability to engineer DNA to develop the first synthetic human insulin, and thereby launching an entirely new biological drugs industry, which has significantly contributed to the global economy and improved the quality of life of diabetic patients. The estimated SynBio market is illustrated in Table 1. An update by BCC Research in 2014 stated that the overall market is expected to grow to US$ 11.8 billion in 2018 with a Compound Annual Growth Rate (CAGR) of 34.4% over the five-year period from 2013 to 2018. These figures and extrapolations are highly uncertain, but growth is expected to be sustained beyond 2018.

Table 1. Global value of the synthetic biology market by end user, 2011-2016 (in USD million) (BCC Research, 2011)

End User	2010	2011	2016	*CAGR% 2011-2016
Diagnostics/pharmaceuticals	902.1	1314.7	5373.3	32.5
Chemicals	125.4	185.0	2783.9	72.0
R&D	73.1	82.8	265.4	26.2
Agriculture	26.7	36.1	307.9	53.5
Energy	19.6	25.8	2108.1	141.2
Total	1146.9	1644.4	10838.6	25.8



**CAGR: Compound Annual Growth Rate; Source: Synthetic Biology. Global Emerging Markets, BIO0bbB, BCC Research; ISBN: 1-59623-834-8, November 2011*

The dawn of SynBio was in January 2000, when two articles were published describing a toggle switch (bistable switch) (Gardner *et al.*, 2000) and a biological clock (the "repressilator") (Elowitz & Leibler, 2000). Both were constructed from circuits of the

same genes, promoters and proteins wired together in different configurations and illustrated the feasibility and predictability of engineering sophisticated functions into biological systems using standardised components. This core concept of SynBio demonstrates the standardisation of parts, systems and design tools that accelerate and empower the engineering of living systems (Carlson, 2010). SynBio has grown to encompass a broad set of technologies, methods and concepts that expand the scope and scale of genetic modifications. Many of these technologies and methods evolved from genetic engineering and include:

I. Genetic part libraries and methods
II. Minimal cells and designer chassis
III. Protocells and artificial cells
IV. Xenobiology
V. DNA synthesis and genome editing
VI. Citizen science (e.g. Do-It-Yourself Biology, DIYBio, which is another important development consequent to accessibility and increased ease of genetic modification.)

Table 2. Examples of categories and uses of SynBio applications

Main Category	Subcategory	Uses
Medical and Veterinary	Therapeutic and preventive	Biological and chemical drugs Vaccines Gene therapy Cell therapy Tissue engineering Antimicrobial agents Probiotics Diagnostics
Personal care products	Cosmetics and personal care products	Skin care Dental hygiene Sun protection
Agriculture	Food and feed	Plant nutrition Plant growth / fitness Animal growth / fitness Food processing Diagnostics
Industrial	Energy & Mining	Novel fuels, e.g. Cellulosic to fuel, Photosynthetic fuel and Fuel upgrading Mineral extraction Desulphurisation of fuels
	Chemicals & Materials	Specialty and bulk chemicals Rubbers and polymers Fibres
Environment	Environment	Remediation Waste treatment Pollution sensors
Others	Information Technology	Biosensors Biochemical encoding of data Nano-devices
	Culture and leisure	Bio art Plants for leisure
	Security	Biodefense

Table 2 illustrates the areas in which SynBio has or might impact daily life.

The following achievements highlight the potential of SynBio through its contributions to medicine, materials, chemistry, food, nutrition, energy, sustainability, waste treatment, and safety. Some significant achievements of SynBio include:

- engineering yeast to manufacture artemisinin – now producing 70 million doses (and growing) per year of the drug to treat malaria in developing nations (Paddon *et al*., 2013, Singer, 2013).
- synthesis of whole yeast chromosomes (Annaluru *et al*., 2014) and bacterial genomes (Gibson *et al*., 2008)
- high-yield production of renewable chemicals using bacteria, algae and yeast including butanol, butadiene, farnesene, isoprene, vanillin and engineered fatty acids for applications in fuels, cosmetics, polymers, rubber, food and health (Chen *et al.,* 2013, Erickson *et al*., 2012)
- engineering of bacteriophage as a rapid assay for food contamination (Schmelcher and Loessner, 2014)
- synthesised gene cassettes, a fairly large-scale genome reengineering with potential to increase photosynthesis in crops (Lin et al., 2014)
- creating an orthogonal biosensor in *Arabidopsis* plants (Antunes et al., 2012)
- engineering of T-cell receptors on immune cells to target and destroy malignant tumours (Restifo *et al.,* 2012)

3.1.2 The future of SynBio

While the advances made by the applications of SynBio highlight the potential of this methodology, the ability to engineer predictable outcomes of biological systems remains embryonic relative to most other fields of engineering. Although scientists can make intentional changes, the effect on a cell's biology may be unexpected. SynBio is a unique field of engineering, because its medium is self-replicating and evolving. The self-replicating nature of SynBio systems and their ability to interact directly with the essential elements of human, animal and plant life raises potential cultural, political, economic, ethical, safety and security challenges. As SynBio is a natural evolution of the field of genetic engineering, SynBio uses and builds upon established mechanisms governing genetic engineering and biological research. Responsible development of SynBio will require continued evolution of governance mechanisms (PCSBI, 2010, EGE, 2009, ERASynBio, 2014). The SCs limited the scope of the analysis in this Opinion to the foreseeable future, acknowledging that its findings should be reviewed and updated as the field evolves.

3.2 Risk governance

3.2.1 Introduction

In this Opinion, SCs are asked to address what the implications are for human and animal health and the environment of likely developments in SynBio and subsequently whether existing health and environmental risk assessment practices of the European Union are adequate for SynBio. Outside the scope of the current mandate are the social, governance, ethical, and security implications of SynBio. However, a brief discussion on the first 3 of these is needed to fully appreciate the understanding of risks of SynBio.

Risk Governance is defined as follows (IRGC, 2008): Risk governance deals with the identification, assessment, management and communication of risks in a broad context. It includes the totality of actors, rules, conventions, processes and mechanisms and is concerned with how relevant risk information is collected, analysed and communicated, and how management decisions are taken. It applies the principles of good governance that include transparency, effectiveness and efficiency, accountability, strategic focus, sustainability, equity and fairness, respect for the rule of law and the need for the chosen solution to be politically and legally feasible as well as ethically and publicly acceptable. The challenge of better risk governance lies here: to enable societies to benefit from change while minimising the negative consequences of the associated risks.

At present, pressures for innovation and economic growth are high, but a parallel activity is needed to avoid/minimise associated potential and perceived health and environmental risks. Responsible innovation is crucial since it contributes to environmental, social, and economic sustainability (Owen *et al.*, 2009, van den Hove *et al*., 2012, UK SynBio Roadmap CG, 2012, Government Office for Science, 2014). SynBio governance needs to adhere to a broader shift that is already occurring in European science and technology policy-making towards 'responsible research and innovation' (RRI). Von Schomberg (Von Schomberg, 2011) has expressed this concept as:

"A transparent, interactive process by which societal actors and innovators become mutually responsive to each other with a view to the (ethical) acceptability, sustainability and societal desirability of the innovation process and its marketable products (in order to allow a proper embedding of scientific and technological advances in our society)"

It is vital to recognise the importance of maintaining public legitimacy and support. To achieve this, scientific research must not get too far ahead of public attitudes and potential applications should demonstrate clear social benefits. Furthermore, the potential benefits of the technology and the risks must not be overhyped creating unrealistic hopes that cannot be fulfilled and/or public anxiety (Balmer and Martin, 2008). Framing a SynBio technology as revolutionary and spectacular not only generates media interest, but may also lead to disproportionate reactions, measures and regulations in the social and ethical sphere. This underlines the continuing importance of accuracy and realism in information provision in all areas of research, and certainly in SynBio (Bubela *et al*., 2012).

A suitable risk governance framework should be identified to encourage responsible innovation. The framework proposed by the International Risk Governance Council (IRGC, 2008 and 2010) is helpful. The risk governance framework includes horizontal scanning coupled to assessment of risks and benefits in an iterative, multi-level and multi-actor process. The process involves interaction with relevant actors from society, including industry, science, NGOs, citizens and takes their interests and values into account (Renn *et al*., 2011, van Asselt and Renn, 2011). Key issues in governance are actors in regulation, application area, boundaries between SynBio and other technologies (nano, information and communication technology (ICT), biotechnology, chemistry, etc.), interrelation between different regulatory systems (protection of workers, environment, medical, etc.), heterogeneity of this technological sector, and the issue of citizen science (risk governance aspects of citizen science are discussed in section 3.4.2). Different levels are political, ethical, legal, professional, scientific, institutional, societal (EGE, 2009). Risk governance can be at the level of authorities, but also "self-governance" should be recognised as an important contribution toward safety, e.g.

Asimolar, J. Craig Venter Institute (Balmer and Martin, 2008, Pauwels *et al*., 2013, Garfinkel *et al*., 2007) especially because more people outside the traditional biotechnology community are expected to be involved in creating SynBio products (Schmidt, 2008).

The SCs conclude that the development and application of SynBio in the European Union will ultimately be determined by public/political acceptability, which may vary according to:

- the scale of the risks in comparison with the perceived or actual benefits
- the potential/ likelihood to control the risks and the trust in this
- a widely accepted means of perceiving the risks, e.g. benchmarking against familiar risks

Elements of acceptance are socio-economic and ethical considerations. Before discussing risk assessment in Section 3.3, these two essential elements in risk governance of SynBio will be discussed. Although biosecurity is an important consideration in this context, it is outside the focus of this Opinion.

3.2.2 Socio-economic aspects

The potential societal impacts of SynBio are many, ranging from energy saving and reduction of CO_2 emissions to novel medicines and consumer goods. Furthermore, it is important to explain their expected beneficial and potentially adverse societal impacts in an understandable and transparent way. SynBio challenges existing consultation mechanisms with regard to their further development.

3.2.3 Other issues: education, skills

Understanding the potential benefits and risks of the development of SynBio processes and products is crucial for the embedding of SynBio activities within our continually evolving societies. This requires developments in education about SynBio for those involved in the development of SynBio products and for those who use them. Consequently, the educational needs range from enabling a basic understanding to the training of scientists developing SynBio products. In the first instance, specific education would be needed at schools and universities and to ensure that workers involved with both manufacturing and disposal/recycling can safely manage or prevent risks.

Another important issue involving education is that there is a growing number of institutions offering graduate level education in SynBio. As the applications of SynBio grow, a common set of tools and techniques means that education and training should reflect this. It is possible that the synthetic biologist of the future will carry out many similar functions that are carried out by engineers in industry today. Currently, there are traditional on-campus courses in addition to the explosion of Massive Open Online Courses (MOOCs), which will enhance, if not partially replace classroom and laboratory work.

3.2.4 Ethical aspects

SynBio is a challenge from an ethical viewpoint because the potential for new products and processes is extraordinarily diverse and hard to predict. Of particular importance is to identify what might be unique in terms of human and environmental exposure.

There are four main generic ethical considerations which are often raised in the context of debates on SynBio. SynBio developments might:

1. Blur the distinction between life and non-life
2. Interfere with nature
3. Widen the gap between have and have-not countries and sectors of society
4. Due to premature use or misuse, lead to serious threats to society (a biosecurity issue, too)

One or more of these issues is frequently raised in response to a major technological development and none of these individual concerns is unique to SynBio although the degree of the hazard together with the many unknowns might be different. Thus, the question is whether the summation of these considerations for SynBio constitutes a 'unique' ethical concern or merely a quantitative rather than qualitative difference.

3.3 Implications of SynBio for risk assessment

In the safety assessment of SynBio, there is high complexity and uncertainty. Risk assessment for SynBio utilising current GMO regulations may soon become challenged (Bubela *et al*., 2012, König *et al*., 2013) and may result in severe restrictions that impede further innovation (Cogem, 2013, Bailey *et al.,* 2012). The uncertain nature of innovative and emerging SynBio technologies (see section 3.7) encourages the development of a transparent, iterative process of risk governance, which includes risk assessment and dialogue among stakeholders including civil society globally as important elements (König *et al*., 2013, SANCO, 2012). Risk assessment must consider hazard identification and characterisation, exposure target, degree of exposure, trends in exposure and number of exposed. Recognising that SynBio evolved from and shares many methodologies and tools of genetic engineering, the SCs consider the assessment of risk guidance documents such as those issued by the GMO panel and/or the GMO unit of the European Food Safety Authority (EFSA) taking into account specific groups of organisms like microorganisms, plants and crops, or animals (Devos *et al*., 2014) and guidance documents for environmental risk assessment of viruses and/or medicinal products (e.g. Baldo *et al*., 2013, CHMP, 2007)[4].

3.3.1 Objectives

The objectives of this Opinion are:

1. The identification of implications of SynBio in terms of risks and, to a limited degree benefits, to human and animal health and the environment. This identification focuses on those developments of SynBio technologies that move beyond the state of the art of genetic modification as practiced about 10 years ago. The first step in risk assessment addresses problem formulation that defines the scope and goals in relation to relevant hazard identification, exposure scenarios, level of uncertainty, acceptable risk, analysis plan and information needs.

[4]Committee for the medicinal product for human use (CHMP). Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products. EMA. [online] 2007.Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003 964.pdf Or Baldo et al.2013 : General Considerations on the Biosafety of Virus-derived Vectors Used in Gene Therapy and Vaccination - See more at: http://eurekaselect.com/117524#sthash.7ZAOTe4G.dpuf

2. Analysis of current risk assessment methodology for SynBio tools, methods and applications. Where such methodologies are considered inadequate, the SCs will provide suggestions for revised risk assessment methods and risk mitigation procedures (including safety locks).

These analyses will be done for the present, short- and medium-term periods considered by the SCs up to approximately 10 years from now. Beyond this period, reasonable estimations of future developments are even more difficult and the SCs suggest revisiting risk assessment methodologies for SynBio at regular intervals.

3.3.2 Potential Hazards

The identification of hazards or potential adverse effects depends on what is to be protected, where to protect it and over what time period.

Table 3: Protection goals and direct and indirect adverse effects

Protection goal	Effects
Humans (workers, general population)	Toxicity, allergenicity, pathogenicity
Animals	Toxicity, pathogenicity
Environment	Plant pathogenicity; adverse effects on biodiversity, ecosystem functions and services

Protection goals, and the set-up of operational specific protection goals, focus on the environmental risk assessment and facilitate the selection of relevant assessment endpoints. This allows for the formulation of testable hypotheses and the selection of measurement endpoints[5]. The following is an example of an appropriate measurement endpoint taken from the environmental risk assessment of plants genetically modified to express toxins that render them resistant to certain target insect pests. During cultivation of an insect-resistant GM plant, it is possible that the toxin produced by the GM plant may exert an unwanted effect on non-target invertebrates. To assess this environmental risk, it is important to set-up appropriate assessment endpoints e.g., the choice of a given species to be tested, and the determination of adequate measurement endpoint allows the formulation of testable hypothesis.

An appropriate measurement endpoint for assessing the effect on non-target organisms is relative fitness (or some component of relative fitness), which is the relative lifetime survival and reproduction of the exposed versus unexposed non-target species. Both lethal and sub-lethal effects observed in non-target organisms are relevant in the assessment of a possible hazard.

For field trials, estimation of ecosystem functions and services[6] should complement experiments conducted on one species in isolation and/or outside its ecological context. Ecological functions such as pollination, biological control, soil functions depend on the number of species, their abundance and different types of assemblages. For example,

[5]Measurable (ecological) characteristic that is related to the valued characteristic chosen as an assessment point.
[6]Ecosystem services: include all services provided by ecosystems, e.g. production of food, fuel, fibre and medicines, regulation of water, air and climate, maintenance of soil fertility, cycling of nutrients. Ecosystems services are distinct from ecosystem functions by virtue of the fact that humans, rather than other species, benefit directly from these natural assets and processes (Millennium Ecosystem Assessment, 2005).

the overall predation rate of a guild of predatory species could be selected as an assessment endpoint in field trials.

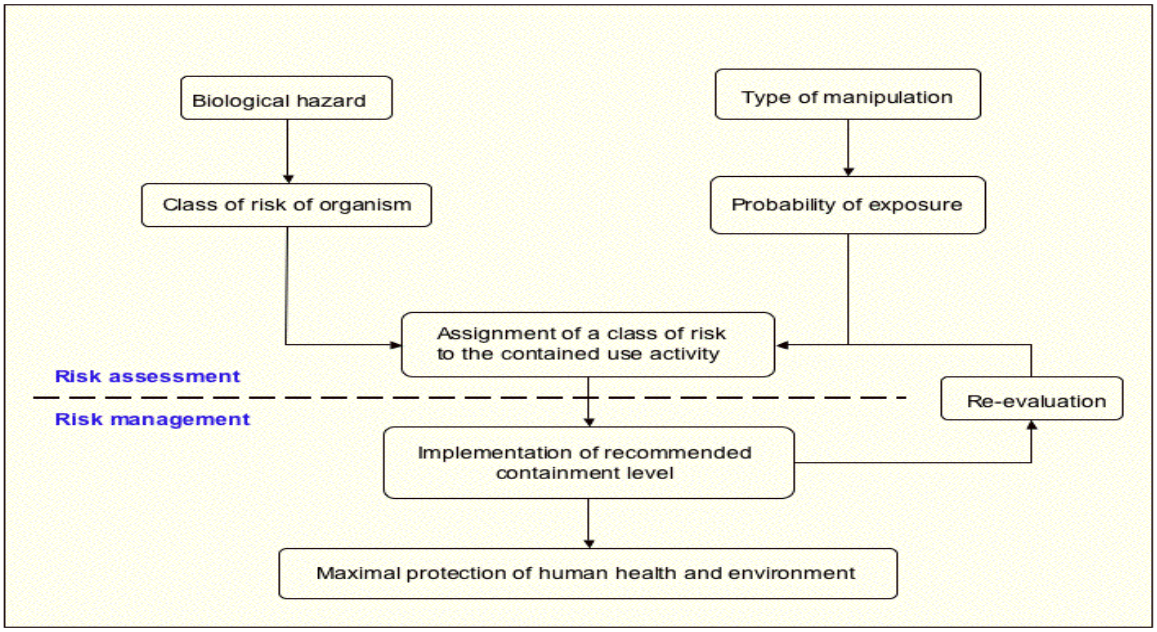
3.3.3 Risk Assessment Method

As defined in the SynBio WG Opinion I: *SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacturing and/or modification of genetic materials in living organisms.*

Possible risks to human, animal end environmental health result from the products that emerge from SynBio methods and tools. For purposes of timely assessing risks of likely developments in SynBio, it would be important to consider novel SynBio tools, methods and products and the potential risks of envisaged SynBio products. Risk assessment only makes sense at the level of the biological system, not for parts in isolation. The SCs identified six novel SynBio developments for consideration in this Opinion. A list of application areas of suitable tools and methods is provided in the Annex III. Risk assessment could be carried out building upon commonly applied principles which are established in Annexes of the Directives 2001/18/EC and 2009/41/EC, which examine the magnitude of potential hazards or adverse effects of genetic engineering on human health and on the environment and the likelihood of the events leading to that hazard (exposure chain). Each of the six novel SynBio developments will be examined for possible risks posing new challenges for their characterisation as well as gaps in the current risk assessment framework. The next two sections outline the principles of risk assessment methodologies of contained use activities and activities involving the deliberate release of GMOs, respectively.

3.3.3.1 Risk assessment of contained use activities (Directive 2009/41/EC)

Figure 1 Biological risk assessment for contained use activities



This section pertains to risk assessment methodology of contained use activities involving GMM (Directive 2009/41/EC) or biological agents (Directive 2000/54/EC). Activities using GMM or biological agents (pathogenic organisms) are often performed by implementing physical barriers or a combination of physical barriers together with chemical and/or biological barriers to limit their contact with the general population and

the environment. Risk assessment of contained use activities consist in the analysis of scientific information to estimate the probability and severity of an adverse effect to determine an appropriate set of containment and protective measures, which are proportionate to the class of risk of the contained use activity. Five major areas include: (see Figure, taken from Belgian Biosafety server[7]). The SCs consider this approach appropriate for the assessment of contained use of SynBio.

1. Identification of biological hazards
2. Determination of the class of risk of the genetically modified or pathogen organism
3. Consideration of the type of activity in terms of probability of exposure to potential biological hazards
4. Assignment of a class of risk to the contained use activity
5. Implementation of recommended containment level (Risk Management)

For the identification of biological hazards and determination of the class of risk of the pathogen organism, classification lists of pathogenic microorganisms were established and are a useful tool for performing a risk assessment[8] (steps 1 and 2). However, some pathogens and most GMOs are not classified into risk groups. The most critical properties inherent to the biological material that should be considered for a risk assessment and assignment to a risk group include:

- Severity of the disease or the infection
- infectivity (virulence of the strain, infective dose, mode of transmission, natural route of infection)
- host range of the micro-organism and spectrum of specificity of target-species
- biological stability
- potential of survival and dissemination in the community or the environment
- availability and effectiveness of prophylactic or therapeutic measures (such as vaccination or antisera, antibiotics, chemotherapeutic agents, taking into consideration the possibility of emergence of resistant strains)

The classification of the biological risk of plant pathogens includes three additional criteria including the:

- prevalence of the micro-organism in the environment
- presence of target-species around the installation or the site of waste disposal
- 'exotic' character of the micro-organism

For GMMs, each element used towards the achievement of the genetic modification should be evaluated as well as the:

- recipient micro-organism
- genetic material inserted (originating from the donor organism)
- vector
- donor micro-organism
- resulting GMM

[7]http://www.biosafety.be/CU/RA_Fiches/Intro_and_menu.html
[8]Reviewed in http://www.biosafety.be/RA/Class/ClassINT.html

Annex III of Directive 2009/41/EC and Guidance notes published in Commission Decision 2000/608/EC describe, in general terms, the elements considered for performing a risk assessment of GMMs.

When considering the type of activity in terms of probability of exposure to potential biological hazards (step 3), not only should risk factors inherent to the biological material be considered, factors associated with the type of operations/modifications should be examined as well. This includes:

- potential for aerosol generation
- scale of the activity
- concentration and volume (e.g. cultures, supernatants)
- type of work proposed (e.g. *in vitro*, *in vivo*, challenge studies, work with laboratory animals, non-standardised manipulations)

The properties inherent to the recipient organism, the genetic material inserted, the vector and the resulting GMO, and the characteristics of the activity are then considered together in a final risk assessment, leading to the assignment of a class of risk of the contained use activity (step 4). The class of risk of the activity may be equivalent to the class of risk of the microorganisms or it may be higher or lower.

The class of risk of the activity defines the level of the recommended containment level. Each level of containment implies the set-up of technical requirements, specific equipment, work practices and other protective measures.

Several biosafety manuals provide a guidance to conduct a comprehensive and thorough risk assessment (some resources are available[9]). It is also important to be aware that a risk assessment should always be carried out on a case-by-case basis and under normal condition and single fault condition.

3.3.3.2 Risk assessment of deliberate release (Directive 2001/18/EC)

Several guidance documents on specific groups of organisms like microorganisms, plants or animals have been issued by the GMO panel and/or the GMO unit of the European Food Safety Authority (EFSA) (Devos *et al.*, 2014). These are based on the principles of risk assessment as outlined in the Directive 2001/18/EC (on the deliberate release of GMOs), which identify six steps in the environmental risk assessment of GMOs:

1. Problem formulation including hazard identification:
   - *Identification of characteristics which may cause adverse effects*
2. Hazard characterisation:
   - *Evaluation of the potential*

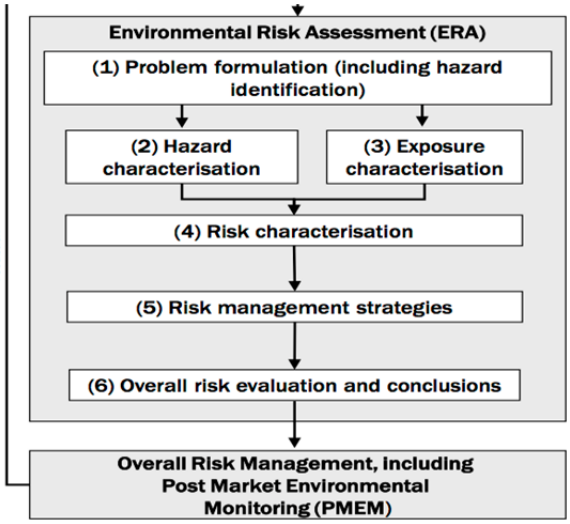


Figure 2. *Six steps within the environmental risk assessment (ERA) and relationship to risk management, including monitoring, according to Directive 2001/18/EC and regulation (EC) No. 1829/2003.*[1]

[9]http://www.biosafety.be/CU/BSL_Ressources/RiskAssessment.html

consequences (magnitude) of each adverse effects, if it occurs

3. Exposure characterisation:
   - *Evaluation of the likelihood of the occurrence of each identified potential adverse effects*
4. Risk characterisation:
   - *Estimation of the risks (magnitude x likelihood) for various endpoints posed by each identified characteristic of the GMO*
5. Risk management strategies
6. An overall risk evaluation

Determination of the overall risk of the GMOs:

For the environmental risk assessment of GM plants, EFSA recommends applying the 6 above-mentioned steps to 7 specific areas of concern:

1. Persistence/invasiveness
2. Gene transfer
3. Interaction with target organisms
4. Interaction with non-target organisms
5. Impact of cultivation, management, harvesting
6. Biogeochemical processes
7. Human and animal health

Not all marketing purposes of GM plants involve cultivation as GMOs can also be approved as food, feed or derived products only. For the risk assessment of GM plants and derived food and feed, the assessment is done on the agronomic and phenotypic characteristics, composition, toxicity, allergenicity and nutritional value. Not only GM plants, but also GMMs are involved in the production of a variety of food and feed. Given the differences in the nature and the level of scientific information required for their evaluation, GMMs and their products have been categorised as follows:

> **Category 1:** Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed (e.g. amino acids, vitamins);
>
> **Category 2:** Complex products in which both GMMs and newly introduced genes are no longer present (e.g. cell extracts, most enzyme preparations);
>
> **Category 3:** Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present (e.g. heat-inactivated starter cultures);
>
> **Category 4:** Products consisting of or containing GMMs capable of multiplication or of transferring genes (e.g. live starter cultures for fermented foods and feed).

Thus far, market authorisation primarily involves GM plants and to a lesser extent GMM. For GMOs and GMMs, the comparative approach appears to work as an internationally accepted baseline for the assessment of risks in the frame of human and environmental health. The comparator is defined as similar organisms produced without the help of genetic modification as defined in Directive 2001/18/EC and for which there is a well-established history of safe use. According to Directive 2001/18/EC, the general principle followed when performing environmental risk assessment is to identify the

characteristics of the GMO and its use which has the potential to cause adverse effects and should be compared to those presented by the non-modified organism from which it is derived and its use in similar situations and related to the added value.

3.4 Risks related to SynBio Tools, Technologies and Methods

3.4.1 Outline of the risk assessment process

It is important to establish when risk assessment 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. Opinion I focused on identifying the relationship between SynBio and GM, and the possibility of distinguishing between the two. It is important to consider 4 distinct reference points as illustrated in Figure 3:

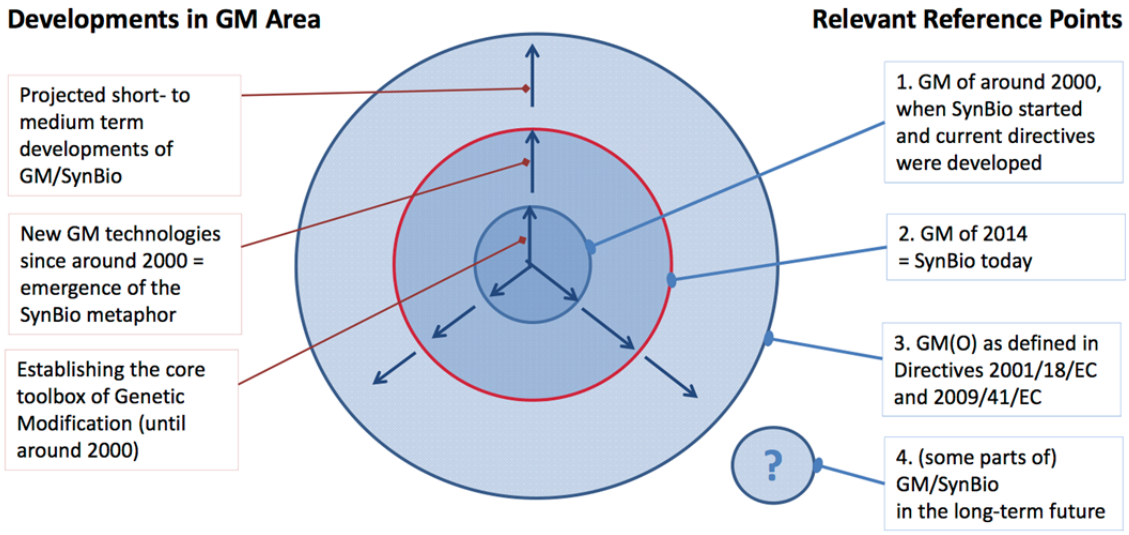


Figure 3. The relationship between SynBio and GM considering 4 different reference points: *1) The first reference point is GM as practiced since ca. 2000, when SynBio began to emerge and the current regulatory framework for GM was developed*[10]*. 2) The second reference point is GM as practiced in 2014/15, 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 practiced 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.*

Depending on the reference point used, the relationship between GM and SynBio is different. For risk assessment purposes, the reference points are potentially relevant because 1) risks that pose new challenges for their characterisation could arise from GM and SynBio developments since the original regulatory framework was established, 2) risks that pose new challenges for their characterisation could be envisaged from

[10]The first GMO Directives were the Directive 90/219/EEC on the contained use of genetically modified micro-organisms and Directive 90/220/EEC on the deliberate release of genetically modified organisms into the environment

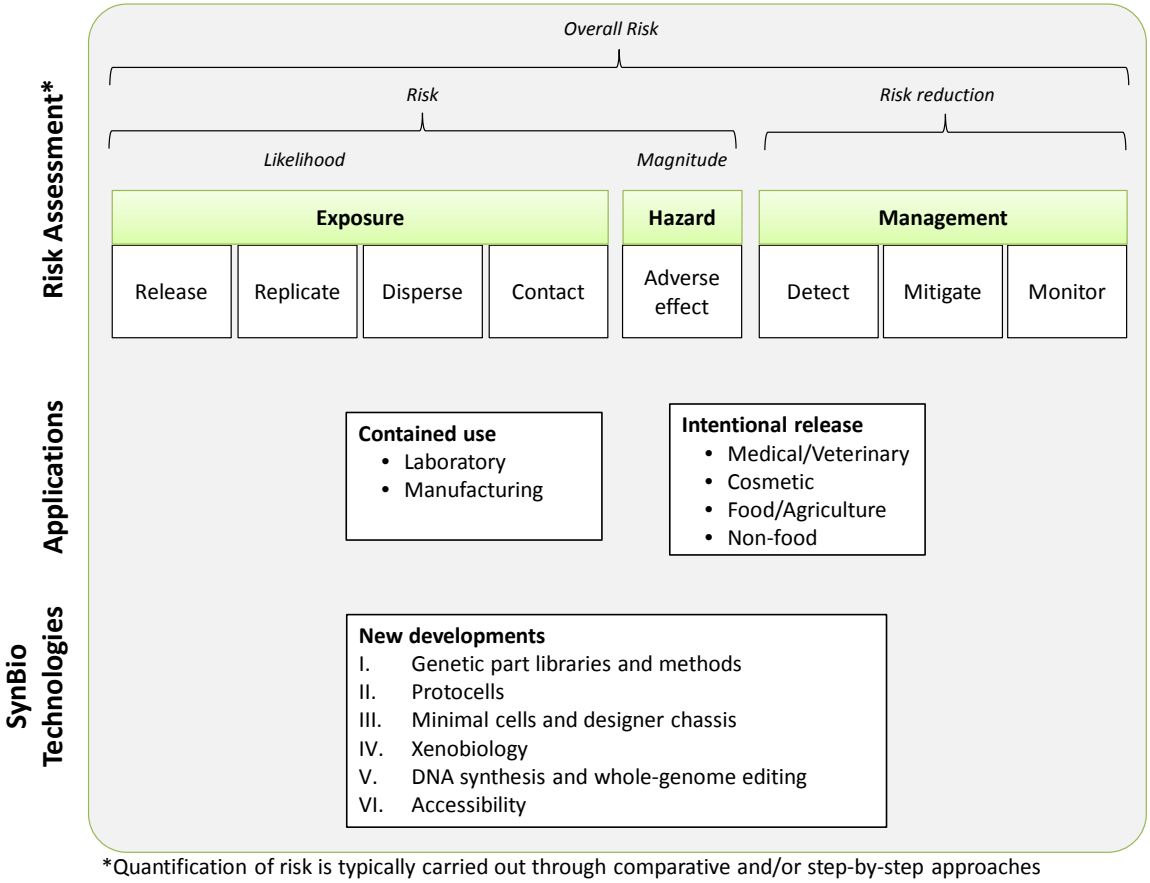
predicted future developments in the same direction, i.e. further acceleration and facilitation of GM, and 3) in the long-term, it is possible that some elements of GM move beyond those reasonably subsumed under the definition of GM in the Directives (Figure 3).

GM and Synbio developments may be either Contained use at laboratory and manufacturing scales or Intentional release including medical, veterinary, cosmetic, Food/Ag, and Non-food. Risk assessment addresses the overall risk with 'risk' and 'risk reduction' components. The former is broken into two additional components, which include Likelihood and Magnitude. Additionally, risk assessment must take into account Exposure, Hazard and Mitigation. Exposure elements include release, replication, dispersion and contact. Hazard is defined as potential adverse effects and Management is defined as prevention, detection, mitigation and monitoring of risks (Figure 4).

The risk characterisation is composed of an estimation of the likelihood of occurrence of adverse effects and the consequences a\of adverse effects. Moreover, risk characterisation should include assessment of uncertainties and an approach to address them. The questions arising from this approach are the following:

1. What is covered under the existing regulations?
2. What are the technical advances over the methods and products covered under existing regulations?
3. What risks are presented by new tools and methods?
4. What gaps or issues are encountered in risk assessment?
5. Are new risk assessment methods recommended?
6. Are new management methods recommended?

Figure 4: An outline of the assessment process



*Quantification of risk is typically carried out through comparative and/or step-by-step approaches

3.4.2 Risks related to SynBio developments

The following SynBio developments are discussed: I. Genetic part libraries and methods, II. Minimal cells and designer chassis, III. Protocells and artificial cells, IV. Xenobiology, V. DNA synthesis and genome editing, and VI. Citizen science will be divided into sections that introduce the development and answer questions 4-6 of the mandate:

I. Genetic parts/circuit libraries and engineering methods

Genetic parts libraries state of the art

The complexity of engineered genetic systems advances were driven by many technological factors ranging from the 1) availability of genome and gene data in databases, 2) improved and more-standardised DNA modification technologies, 3) advanced tools and resources for measuring and selecting modified strains, 4) computational and analytical tools for designing complex genetic systems, and 5) greater public and private investments in cutting-edge genetic engineering technologies. Engineered genetic systems may be composed of many tens of different parts recombinant, mutated or synthesised DNA parts[11]. To engineer these complicated genetic systems, there are electronic and physical repositories of genetic elements often

[11]Commissao Tecnica Nacional de Biosseguranca, Technical Report No. 3287/2012 - Commercial release of yeast (Saccharomyces cerevisiae) genetically modified to produce farnesene by strain Y5056 - Case No. 01200.003977 / 2011-56. 2012. http://www.ctnbio.gov.br/index.php/content/view/17454.html

called “genetic parts libraries” which contain genes and DNA fragments with characterised properties and functions maintained in a form that facilitates faster search, retrieval and assembly into novel engineered genetic systems. Some of these libraries have thousands of parts, which are publicly accessible[12].

The creation and operation of repositories of DNA, genetic material or biological tissues and organisms is not a novel development. Non-profit and for-profit entities including the Coli Genetic Stock Center and the American Type Culture Collection[13] have maintained repositories and sold genetic material for decades. The main advance in SynBio is the degree to which the genetic material is designed and engineered for interoperability and speed of assembly, which allows more complex systems to be constructed. SynBio libraries characterise the functional properties of each element in the library in great detail and precision and deploy advanced information technologies to ensure that the information is available to designers and users. This information is intended to accelerate biological design, similar to how computer-aided design accelerated other engineering fields. In practice, detailed characterisation of genetic elements is difficult and labour-intensive and many of the parts in current SynBio parts libraries remain poorly characterised, except at the most basic level of biochemical function. Thus, by now genetic engineering remains more dependent on empirical trial-and-error than other contemporary fields of engineering (Gardner, 2013, Gardner and Hawkins, 2013).

Question 4: *What are the implications for human and 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?*

The continued advancement of genetic parts libraries has the following implications for human, animal and environmental health and safety:

Better functional information. The availability of increasingly detailed, precise and accurate information on the biological function of parts in genetic libraries will improve the effectiveness of risk assessment as it pertains to appraisal of potential hazards to humans, animals or the environment. This information will be particularly valuable for risk assessment conducted for prospective SynBio research activities to determine the appropriate containment level.

Working with parts of unknown function. Any library of genetic parts will contain DNA elements of unknown function. Such parts may be in-queue for characterisation, or they may simply be carried along in the genomes of donor organisms. Research on DNA of unknown function has been conducted in molecular biology for many years already and therefore does not present novel challenges for risk assessment. However, large-scale construction of SynBio libraries and using them without detailed characterisation of individual parts may increase the frequency of use of uncharacterised components. Thus, managers of SynBio libraries should establish safety protections consistent with hazards presented by DNA of unknown function. Typically, this means the adoption of containment levels based on the properties of the source organism, until a lower level of

[12]Registry of Standard Biological Parts. http://parts.igem.org/Main_Page. Accessed 1 October 2014.
[13]Coli Genetic Stock Center. http://cgsc.biology.yale.edu/. Accessed 1 October 2014; American Type Culture Collection. http://www.atcc.org/. Accessed 1 October 2014.

hazard can be demonstrated. These safety practices are currently applied in biological and SynBio research.

Emergent properties. Genetic parts libraries are intended to facilitate the construction of more complex genetic systems, i.e. systems with more components and more complex interactions between components. The function of these systems may be "emergent," i.e. they arise from the interactions of the parts with each other. Emergent functions may include conditional, time-varying and non-linear (non-proportional) behaviours (Guet *et al.*, 2002). The current Directives 2001/18/EC and 2009/41/EC for risk assessment consider these emergent properties by requiring an assessment of the proposed or realised GMM/GMO, in addition to an assessment of the properties of component parts. Notably, the emergent properties may present new challenges in predicting or testing for risks and in the identification of appropriate comparator organisms. This point is specifically addressed in the following paragraphs.

Question 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?*

The activities and products involving the creation and introduction of new nucleic acid molecules and/or hereditary material into cells are subject to the provisions of existing EU GMO Directives. Directive 2009/41/EC (contained use) requires a case-by-case risk assessment for the classification of activities involving the use of GMMs into one of four risk categories (negligible, low, medium and high) and enforcement of appropriate containment and protective measures. Directive 2001/18/EC (deliberate release) mandates a case-by-case process to approve or deny a permit for the intentional release of a GMO into the environment on the basis of an environmental risk assessment. The comprehensive nature of the case-by-case risk assessment and mitigation procedures of the Directives is appropriate and adequate to manage the risks of SynBio activities and products associated with genetic parts libraries.

Better functional information: More functional information promises to decrease the uncertainties of potential hazards of genetically engineered organisms using those parts. This information includes the basic biochemical properties of the nucleic acids and their protein or molecular products as well as data on the interactions of those molecules with other biochemical elements. In addition, the SynBio community is continuously improving the reliability of functional information and its accessibility. This information may ultimately be incorporated into models of biological function used to predict potential failure modes. Thus, SynBio outcomes are likely to enhance safety and risk assessment.

Working with parts (DNA elements) of unknown function: The construction of parts with unknown function poses no risks other than those previously encountered in genetic engineering. Research on parts of unknown function demands diligent application of established risk assessment methodologies, which lead to the assignment of a containment level and protective measures that offer maximal protection for human health and the environment. These risk assessment procedures are currently applied when handling biological agents (e.g. Directive 2000/54/EC or Laboratory Biosafety Manual, 3rd edition (World Health Organization, 2004; CDC/NIH: Biosafety in Microbiological and Biomedical Laboratories) or GMM (Commission Decision 2000/608). The risk factors inherent to the biological material including GMO or biological agents and

risk factors associated with the type of operations/modifications are considered. The latter include an assessment of the potential for 1) aerosol generation, 2) the scale of the activity, 3) the concentration and volume used, e.g. cultures, supernatant, and 4) the type of work proposed, e.g. *in vitro*, *in vivo*, challenge studies, work with laboratory animals, non-standardised modifications. The classification of activities involving parts with unknown function should consider the biological risk class of the donor organisms, and each element used towards the construction of the library, including the recipient organism, genetic material inserted and vector.

Emergent properties of complex genetically engineered organisms: These organisms present novel issues for the application of current risk assessment. While the SCs conclude that the methodology of risk assessment Dir 2001/18 and 2009/41/EC is still appropriate, they also conclude that the application of this methodology may require novel tools, e.g. for predicting emergent properties of complex genetic systems. Firstly, the tools for predicting emergent properties of complex genetic systems may not be sufficiently reliable or may not be available to risk assessors, which limits prediction and may impair the ability to accurately identify, test for or mitigate potential hazards. Secondly, the genetic distance between a SynBio organism and a comparator organism used in risk assessment may be large and may be exaggerated if the comparator organism selected for testing is a non-GMO organism, as is the current practice. With greater genetic distance comes greater potential for unexpected emergent properties and failure modes due to a higher number of interactions between parts. However, by now even the most complex organisms created by humans are genetically close to their non-GMO parents with engineered organisms sharing 99-100% of their DNA with their parent organism. Additionally, understanding the biological functions and the tools for simulating their function in parallel to the advances in the complexity of the organisms created (Lerman JA, *et al.,* 2012), which indicates that tools for predicting risks will improve. Third, SynBio organisms are tested more thoroughly as they advance toward industrial scale (as explained for GMOs in Opinion 1). This is a necessary part of product design and development, which ensures that resources are wisely invested (Gardner, 2013). Testing generally involves thousands of tests and millions of dollars of investment, generating a sizeable data set and experience base that supports risk assessment, which should be shared with risk assessors.

The SCs conclude that the current methods outlined in Directives 2001/EC/18 and 2009/EC/41 are appropriate and adequate for the management of the risks of SynBio activities and products associated with genetic part libraries. However, incremental advances in the knowledge base and tools for risk assessment are recommended by the SCs to ensure the highest quality risk assessment.

Question 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?*

Present methodologies are appropriate and adequate for assessing the potential risks of activities and products associated with genetic parts libraries of SynBio to ensure high-level protection. The SCs suggest that improvements can be made to maintain the highest quality of risk assessment, which will ensure continued safety proportionate to the true level of risk, without inhibiting SynBio innovation and progress.

The SCs recommend the following:

1. Support research that 1) characterises the function of biological parts, 2) develops computational tools to predict emergent properties of SynBio organisms and their potential failure modes and 3) broadly disseminates knowledge and trains scientists.

2. Streamline and standardise across EU member states the methods for submitting genetic modification data and genetic parts information to risk assessors, which should be transparent and available to all stakeholders.

3. Encourage the use of GMOs with proven safety records as acceptable comparators for risk assessment, i.e. the baseline state of safe organisms can advance with the complexity of new modifications. Reliance solely on non-GMO organisms, as opposed to GMOs with a history of safe use would prevent the advance of baseline risk assessment controls. In contrast, use of GMOs with a record of safety may better reflect the current understanding of risks.

4. Support additional research and debate towards the development of sufficiently sophisticated risk assessment tools to match the advances in technology assessed, to avoid an imbalance between RA and technology that might negatively impact economic and health benefits of the technology and jeopardise the quality of safety protections.

5. Support a Biosafety clearinghouse on bioparts, devices and systems to support risk assessment of genetic circuits generated with biological parts, devices and systems. The SCs suggest sharing relevant information about specific parts, devices and systems with risk assessment practitioners.

The above recommendations aim to ensure that risk assessment methods for genetic parts libraries, and their associated engineered organisms, will continue to advance in step with the field of SynBio.

II. Minimal cells and designer chassis

Minimal cells and designer chassis are an alternative approach to the construction of industrial microbes. The approach involves the concept of a minimal genome: the minimum number of genes required to support basic life (Mushegian, 1999). The objective is to minimise the metabolic burden on the cell, so the remaining cellular energy can be directed toward the manufacture of a desired industrial product, such as an industrial chemical or a pharmaceutical (Pyne *et al*., 2011). Minimising the number of components required to support biological synthesis from synthetic DNA circuits or genomes may also simplify control of the function(s).

The only minimal genome so far used as a starting point is the organism with the smallest known genome that can be cultivated under laboratory conditions, the bacterium *Mycoplasma genitalium* (Gibson *et al*., 2008, Glass *et al*., 2006). Precisely 100 of the 482 *M. genitalium* genes were deemed non-essential. Deletion of these genes resulted in a strain with improved growth rates.

For biotechnology applications, reducing the genomes of *Escherichia coli* (*E. coli*) and other minimal risk (BSL-1)[14] biotechnology workhorses is more useful than reduced-genome *M. genitalium* (Jewett & Forster, 2010). Many projects have aimed at reduction of the size of the *E. coli* genome. For example, strains with deletions removing 15% of the genome were not only viable, but they also improved the properties for applications in molecular biology (Posfai *et al*., 2006).

Modules in chassis strains

The SynBio concept envisages the idea of introducing parts or parts arranged in modules or sub-systems into chassis strains. Thus, having removed "inefficient" genetic material, more efficient material can be added back. This is essential to future production strains as natural microbes were not, of course, designed for the rigours of industrial process. So apart from the core genetic material for the production of the new material (e.g. fuels, chemicals or polymers, or pharmaceuticals), designer "modules" may be introduced with specific functions in mind. Some examples are:

- Specific safety features, such as synthetic counting circuits for programmed cell death after a defined environmental retention time (Wright *et al*., 2013)
- "Robustness": e.g. tolerance to excess nutrient levels, extremes of pH, extremes of temperature, toxic metabolites (e.g. acetic acid in *E. coli*), high shear stress
- Elimination of biofilm formation
- Elimination of catabolite repression
- Resistance to product toxicity and improved solvent tolerance
- Countering gene dosage limitation in plasmid-free strains
- Artificial cellulosomes for the contemporaneous decomposition of lignocellulose

Irrespective of the risk group of the recipient chassis strain, and according to current risk assessment principles of GMM, the assessment of the resulting bioreactor-ready production strain, obtained by the introduction of different modules, necessitates an evaluation of each element that has been used towards its achievement, thereby including an assessment of the genetic material inserted, the donor organism and the method used for insertion of the genetic material. If the genetic modification is achieved without inserting material from outside the cell (directed evolution, marker-assisted breeding), it does not fall under the GMO regulations.

The minimal cell approach may be particularly helpful for redesigning those microbes, which, although possessing biotechnology potential, have poor genetic tools available e.g. *Rhodococcus*, which is full of promise, but limited in application.

Lessons from endosymbionts

Endosymbionts are organisms that live within the body or cells of another organism. The phenomenon of genome downsizing that has been observed in endosymbionts (Moya *et al*., 2008) may act as a model for the study and understanding of engineering minimal

[14]BSL = Bio-safety Level, and refers to the level of risk posed by a microorganism, virus or prion.
Risk group 1: Agents are not associated with disease in healthy adult humans.
Risk group 2 (low to moderate risk): Agents are associated with human disease of mild to moderate severity. There are often preventative or therapeutic interventions available.
Risk group 3 (moderate to high risk): Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions *may* be available.
Risk group 4 (extreme risk): Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are *not usually* available.

cells: here “minimal life” is based on the hypothesis that genomes must retain essential genes that are involved in housekeeping functions, and a minimum number of metabolic transactions for cellular survival and replication. Their parasitic or symbiotic lifestyles are programmed through reduced genomes when compared with their closest free-living relatives. Whilst endosymbionts may not be useful as industrial organisms, they may therefore offer fundamental insights into the process of genome minimisation and how the process of minimisation itself may influence risks.

Question 4: *What are the implications for human and 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?*

Minimising the number of components required to support biological synthesis from synthetic DNA circuits or genomes may also simplify control of the function(s). Irrespective of the risk group of the recipient chassis strain and according to current risk assessment principles of GMM, the assessment of the resulting bioreactor-ready production strain (obtained by the introduction of different modules) necessitates an evaluation of each element that has been used towards its achievement, thereby including an assessment of the genetic material inserted, the donor organism and the method used for insertion of the genetic material.

Question 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?*

It is possible to use existing methodologies because minimal cells do not raise different type of concerns compared to the wild type organisms they are derived from.

Question 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?*

No change in existing methodologies is considered necessary.

III. Protocells and artificial cells

Introduction, state of the art and technical advances

In protocell research, 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). 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).

The results of genome-level engineering are based on natural genomes, rather than the design of *de novo* organisms (SBSTTA, 2014). Currently, the systems constructed by “bottom-up” approaches are not alive, but are chemical vesicles, called ‘‘protocells’’ (Rasmussen, 2009). Research in this area is vibrant, but far from having commercial applications. The evolution from a protocell to a truly autonomous artificial cell capable of growing, reproducing and evolving has not yet been created and it is expected that

this will not be possible for many years (COGEM, 2013). Some basic systems have been developed, including the demonstration of chemical copying of RNA templates inside protocells (Adamala and Szostak, 2013; Blain and Szostak, 2014), but more sophisticated artificial cells with complex functionalities (especially the capacity of robust self-replication) are not yet available. It is very hard to predict how soon these techniques will be perfected or when such applications might be considered ready for wider dissemination (SBSTTA, 2014). However, it was predicted in 2009 that a protocell is first realised as a mandatory symbiont to natural forms of life before it is able to survive as an artificial cell. This type of symbiont was reported (Lentini *et al*., 2014) as a result of the integration with *E. coli* a riboswitch, coded by a DNA-template contained in a phospholipid vesicle and controlling the synthesis of the pore-forming protein alpha-hemolysin. This protocell releases a chemical messenger molecule in presence of theophylline only. This results in a new sense-and-report function that otherwise would only have been achievable with genetic engineering.

Question 4: *What are the implications for human and 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?*

Currently, protocells are non-living vesicles and will likely be confined to the laboratory for the near to medium-term. Although the objective is for such cells to replicate, it is not yet possible. Therefore, dispersion is not possible because of the lack of cell viability. However, accidental exposure of humans to protocells in the laboratory may occur. Integration of protocells into living organisms and future development of autonomous protocells warrants the examination of possible routes of exposure and adverse effects. Present developments in protocell research are likely to fall within a regulatory framework covering chemicals rather than within the current GMO regulatory framework (Pauwels *et al*., 2013). Risks related to protocell research are not higher than other risks in biological and chemistry laboratories (Bedau *et al*., 2009), because the current state-of-the-art research does not create novel, viable artificial cells. The situation would need to be reviewed when major progress towards the creation of viable artificial cells is foreseeable.

Question 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?*

Existing available methodologies are appropriate for protocell risk assessment. However, it will be important to select the correct methodologies from the chemical and biological fields, because protocells fall in between chemistry and biology. In the future, the exposure to autonomous artificial cells that survive in the laboratory and in the environment might be possible. Those cases would require an additional risk assessment which might be complicated if there are no natural reference organisms or data on interactions with other organisms and the environment.

Two situations can be distinguished:

1) Protocells that depend on interactions with natural cells

Integration with natural cells was demonstrated (Lentini *et al*., 2014), which illustrates that, although protocells are not alive, they can be engineered to intimately interact with living cells and enhance overall system functionality. A key issue is the potential

outcomes of research in which interactions are established with natural organisms. In these cases, the host range should be identified to avoid unlikely, but not impossible, infections by protocells, especially if they are different from natural cells (Schmidt *et al*., 2009). Importantly, it is necessary to determine the specificity of symbiotic interactions between protocells and natural cells and to determine the outcome of unforeseen interactions of other cells with protocells.

2) Autonomous protocells

A truly autonomous artificial cell capable of growing, reproducing and evolving has not been created. An autonomous protocell would be a self-assembling and self-reproducing chemical system with the properties of containment, metabolism and programmability, designed to survive and reproduce in a changing environment (Bedau *et al*., 2009). If autonomous artificial cells are created in the future, the genetic information that controls internal functioning might mutate. Thus, a population of protocells with different genetic information could undergo selection and new protocells could arise (Bedau *et al*., 2009), which is an example of unpredictable emergent properties of protocell research (Rasmussen, 2009). If protocell research progresses towards autonomous, replicating chemical systems, which react dynamically to changes in their environment, hazardous properties of these cells should be assessed in the context of their intended use (contained use activity versus applications involving intentional release into the environment). Additionally, allergenicity, pathogenicity, biological stability, etc. must also be considered (Bedau *et al*., 2009). The framework for risk assessment of these cells should draw on, but not necessarily be confined to, the methodology used for GMO risk assessment.

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

For protocells that depend on interactions with natural cells, novel biological functions can be designed without altering the DNA of these target organisms, demonstrating that it is crucial to screen SynBio subfields (Section 4.4.3) and combinations thereof to identify unknown hazards. For future autonomous, replicating protocells, it is likely that a case-by-case approach drawing upon a combination of existing frameworks including GMO and regulatory frameworks for chemicals and drugs will be required. The assessment of the resulting autonomous cells obtained by the introduction of different genetic parts, synthetic organelles and biochemicals necessitates an evaluation of each individual element as well as the interactions between them and with the environment.

IV. Xenobiology

Xenobiology (XB) is the design, engineering and production of biological systems with non-canonical biochemistries and/or alternative genetic codes. XB is a subfield of SynBio that includes the following research and innovation areas:

- Xeno nucleic acids (XNA)
- Expanded genetic alphabet: alternative base pairs
- Novel polymerases and ribosomes
- Genetic code engineering
- Non-canonical amino acids (nc-AA)

There are three main aims in XB:

- to understand the origin of life, why life has evolved the way it has and not differently;
- to produce economically interesting organisms or compounds with useful functions;
- to implement new types of biocontainment, impeding horizontal gene flow between natural and engineered organisms

Xeno nucleic acids (XNA)

The chemical backbones of DNA and RNA are deoxyribose and ribose, respectively, and appear to be highly conserved biochemical structures in nature (Eschenmoser 1999, Pace 2001). When another chemical structure is used as a base-carrying backbone, the abbreviation of the resulting nucleic acid changes, e.g. to HNA (hexose), CeNA (cyclohexenyl) or TNA (threose) (Chaput *et al.,* 2003, Ichida *et al.,* 2005, Kempeneers *et al.,* 2005). The collective term for all nucleic acids that are not DNA or RNA is XNA, where the X refers to xeno (foreign) (Marliere, 2009).[15]

Expanded genetic alphabet: alternative base pairs

The two natural base pairs in DNA are A-T (A-U in RNA) and C-G. These base pairs match because their chemical architecture and the number of hydrogen bonds fit together (A-T has two and C-G has three hydrogen bonds). C and T are pyrimidines and A and G are purines. Additional base pairs can be synthesised and incorporated into DNA (or XNA)(Malyshev, A. *et al*, 2014). To extend the genetic alphabet, the new base pairs need to match each other with high accuracy and discriminate against other existing bases for correct replication. For each added base pair, the genetic alphabet grows by 2, in the special case of a self-pairing base it would grow by 1.

Novel polymerases and ribosomes

In most cases, natural polymerases and ribosomes (and other nucleic acid interacting proteins) do not work on XNAs and nucleic acids with expanded alphabets. To allow for replication, transcription and translation, the nucleic acid cell machinery must be adapted to operate on these novel nucleic acids. Encoded synthesis of unnatural biopolymers by polymerase engineering has been demonstrated (Pinheiro *et al.,* 2012).

Genetic code engineering

In most species, genetic information is translated into amino acids according to the so-called universal or standard genetic code[16]:

```
AAs     = FFLLSSSSYY**CC*WLLLLPPPPHHQQRRRRIIIMTTTTNNKKSSRRVVVVAAAADDEEGGGG

Starts = ---M---------------M---------------M----------------------------

Base1   = TTTTTTTTTTTTTTTTCCCCCCCCCCCCCCCCAAAAAAAAAAAAAAAAGGGGGGGGGGGGGGGG

Base2   = TTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGG
```

[15]Sometimes the term 3NA is used for third-type nucleic acid.
[16]http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi?mode=c

```
Base3   = TCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAG
```

However, researchers have identified codes different from the standard code, for example, the vertebrate mitochondrial code (changes marked in red)

```
AAs     = FFLLSSSSYY**CCWWLLLLPPPPHHQQRRRRIIMMTTTTNNKKSS**VVVVAAAADDEEGGGG
Starts = ----------------------------MMMM---------------M------------
Base1   = TTTTTTTTTTTTTTTTCCCCCCCCCCCCCCCCAAAAAAAAAAAAAAAAGGGGGGGGGGGGGGGG
Base2   = TTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGGTTTTCCCCAAAAGGGG
Base3   = TCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAGTCAG
```

To date, at least 25 different natural codes were discovered and researchers have engineered additional codes resulting in genomically recoded organisms (GRO) (e.g. Lajoie, 2013a, Lajoie, 2013b). Theoretically, the code that is currently based on base triplets can be expanded based on four bases (quadruplets) or more, or decreased to two bases (duplets).

Non-canonical amino acids (nc-AA)

Amino acids used for peptide/protein synthesis (proteinogenic amino acids) are called canonical amino acids. In most species, there are 20 amino acids, but in some species additional amino acids are found, either pyrrolysine known from methanogenic organisms and other eukaryotes or selenocysteine known from many non-eukaryotes and some eukaryotes. Through bioengineering, a number of additional, ncAA can be incorporated into proteins, where the resulting protein is an alloprotein. Notably, the incorporation of ncAAs refers to the translation process of mRNA to amino acids. It does not cover the wide number of post-translational modifications found in natural organisms.

Question 4: *What are the implications for human and 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?*

The use of non-standard biochemical systems in living cells, e.g. XNA, alternative base pairs, etc., has implications for risk assessment and biosafety. New variants must be tested for risk to human health and the environment and the xenobiological systems could be engineered to allow for improved biocontainment, e.g. the so-called ‘genetic firewall’ that aims to avoid the exchange of genetic material through horizontal gene transfer or sexual reproduction between the XB and natural organisms.

Question 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?*

It is important to assess xeno-systems for potential toxicity (Schmidt and Pei, 2011). Attention must be focused on determining how different forms of (semi) xenobiological systems behave in a natural environment, i.e. competitiveness. The assumption is that xeno-systems would not survive due to their particular auxotrophy, but contained trials are required.

XB experiments are mostly at the proof-of-concept and basic science level with many years ahead to produce commercially useful applications. The techniques used are similar to those used in genetic engineering. According to the Directives 2001/18/EC [17] and 2009/41/EC, XB could be covered under the definition of GMO. However, it is possible that xenobiological products may not be defined as GMOs, when a result of directed evolution experiments, e.g. as a point of reference see to the incorporation of 5-chlorouracil as 4th base (Marliere, 2011). This is a strong focus on the process of creating the engineered organisms, with a special focus on determining whether novel heritable material was produced OUTSIDE the cell and then INTRODUCED to the cell. At the time when the current regulatory framework was developed, non-standard biochemical forms of nucleic acids were not envisioned. The GMO definition does not specify the biochemistry of heritable material, i.e. it does not state that GMOs may only contain **D**NA. Existing risk assessment methodologies are appropriate to assess the risk of xenobiological organisms, because they are considered GMOs, according to the definition in Directive 2009/41/EC and 2001/18/EC.

Question 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?*

Although current risk assessment methodologies are appropriate to assess xenobiological product risks, there is a need to generate supporting information and data to enable the successful deployment of these methodologies. The existing pool of knowledge of risk characterisation of GMOs cannot entirely be transferred to XB. For example, evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to virus, diseases or predation, toxicity that are included in established risk assessment methodologies for canonical biological systems, may not be compared to xenobiological systems. A scientific basis for the characterisation of xenobiological systems must be established for risk assessment to yield a meaningful outcome, i.e. general data on the evolutionary fitness, ecological competitiveness, degree of horizontal gene flow, susceptibility to virus, diseases or predation, and toxicity of xenobiological systems, and this should happen independently of the particular biological application.

V. DNA synthesis and genome editing

DNA synthesis generates canonical biological systems with entire designer genomes such as the construction of Poliovirus by mail-ordered DNA sequences (Cello *et al*., 2002a, Cello *et al.,* 2002b), first *de novo* synthesis of a DNA virus (bacteriophage phiX174) (Smith *et al.,* 2003), recovery of the '1918' influenza virus from preserved tissues of victims (Basler *et al.,* 2001, Tumpey *et al.,* 2005), assembly of chemically synthesised DNA segments into bacterial genomes, e.g. for *Mycoplasma genitalium* and *M. mycoides* (Gibson *et al*., 2008, Gibson *et al.,* 2010) and the synthesis of a complete 'eukaryotic'

[17] A 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. Techniques of genetic modification referred to in (2001/18/EC) 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.

chromosome arm (Dymond *et al.,* 2011, Muller *et al.,* 2012). In contrast to the traditional transfer of genetic material from one organism to another, with minor modifications, DNA synthesis can be used for both: generation of highly modified, even newly designed sequences as well as for replication of natural or slightly modified sequences. For example, the first eukaryote chromosome synthesis in yeast was modified to differ from its natural counterpart by replacing all TAG stop codons by TAA stop codons with the ultimate aim of using the TAG codon to encode for novel amino acids in an extended genetic code, and removing introns and transposons that were considered functionless historical legacy material, and redundant tRNA loci (Annaluru *et al.*, 2014). In addition, the synthetic genome contained engineered recombination sites flanking every gene, which allowed systematic random scrambling of the genome while maintaining the integrity of the coding sequences to create a large pool of modified organisms to screen and select for desired properties. An important consequence of the availability of large-scale DNA synthesis methods is the reduced reliance on the availability of physical DNA constructs for genetic modification. Traditionally, DNA for modification would be cloned into plasmid vectors and transferred between laboratories in physical form, but now it is possible to exchange sequence information electronically and synthesise any required DNA. Once low-cost desktop DNA synthesisers become widely available, the availability of DNA constructs will substantially increase the accessibility of genetic modification techniques.

Genome editing, using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system, Transcription activator-like effector nucleases (TALENs), or zinc-finger nucleases (ZNF), enables the rapid introduction of targeted genetic modifications in existing genomes (Esvelt and Wang, 2013, Sander and Joung, 2014). These techniques can be applied in a wide range of higher organisms (plants, animals), accelerating their genetic modification considerably (from many months to a few weeks in the case of mice) and facilitating the modification of non-model organisms. A large number of modifications may be introduced in parallel. New techniques may also be used in a multiplexed fashion, allowing the simultaneous generation of large numbers of variants that can then be screened or selected for desired properties (Dalia *et al.*, 2014). This study does not describe the use of engineered nucleases, but uses a method relying on the accelerated evolution in naturally competent bacterial species (thus, relying on natural competence and transformation). In contrast to most traditional methods for genome alteration, new technologies for targeted genome editing do not require drug-selectable markers and do not leave behind genomic ‘scars’ associated with the modification. Additionally, they do not require the permanent introduction of extraneous genetic material. As a consequence, in some cases, the resulting modifications are indistinguishable from naturally occurring mutations or organisms derived by chemical mutagenesis. (Araki *et al.*, 2014).

Question 4: *What are the implications for human and 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?*

The new technologies for DNA synthesis and genome editing accelerate genetic modification and increase the range and number of modifications that are easily possible. In addition to the increased speed of modifications, which might pose risk assessment challenges, the following aspects need special consideration:

- Targeted genetic modifications in higher animals are now possible within a single generation by direct genome editing of zygotes.
- Many of the new methods allow multiplexed genetic modifications, which affect a large number of loci at the same time. The resulting organisms are screened or selected afterwards, but their risk is not necessarily assessed individually.
- The number of genetic modifications introduced in parallel by large-scale DNA synthesis and/or highly-parallel genome editing increases the distance between the resulting organism and any (natural or modified) organism to which it could be compared for risk assessment purposes.

Question 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?*

The acceleration of the genetic modification process by advances in synthetic genomics and DNA synthesis calls for more efficient procedures for risk assessment, especially where genetic modifications are introduced in a highly parallel manner. Other relevant aspects related to the applicability of a comparative risk assessment methodology are discussed in section 3.4.2.

Question 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?*

The required acceleration of the risk assessment process might be achieved by identifying appropriate groups of genetic modifications that can be assessed in a "categorised" manner, thus alleviating the need for individual risk assessment in the case of highly parallel and multiplexed genetic modifications. These categorisation protocols should take into account risks potentially arising from the synergy of combined modifications. For example, if a library of organisms carrying variants of a metabolic pathway, with different enzyme variants and promoter strengths, is created, risk assessment might not be required for each individual modified organism, but only for the library as a whole. The appropriate units for this kind of categorisation would need to be justified by further research, before this approach could be implemented in practice.

VI Citizen science

Do-It-Yourself Biology (DIYBio)

As SynBio advances, its methods, equipment and technologies will be cheaper, simpler and easier to use. Thus, SynBio will likely foster citizen science, i.e. attracting DIY biologists into a field traditionally reserved for highly trained professionals (Bennett *et al.*, 2009, Pedersen & Phillips, 2009). DIY research societies founded in many scientific disciplines, e.g. computer science, astronomy, etc. include DIY biologists as "individuals who conduct biological experiments as an avocation rather than a vocation" (NSABB, 2011). These individuals may have some or no formal training in life science, but are highly curious about the science and/or methods. It is estimated that there are thousands of self-appointed DIY biologists worldwide interested in DNA sequences, microbial screening, environmental monitoring, applications for health care and energy,

to name a few (You, 2010). DIY biologists are increasingly organised into formal member groups that aim to enable[18]:

- Open access: promote citizen science and decentralised access to biotechnology
- Transparency: emphasise transparency, the sharing of ideas, knowledge and data
- Education: educate the public about biotechnology, its benefits and implications

The most prominent groups in citizen science are www.DIYbio.org in the USA and www.DIYbio.eu in Europe, which has more than 2000 registered members in more than 20 regional groups in 30 countries (Scudellari, 2013). Currently, most DIYbio activities are focused on teaching members basic knowledge via seminars, workshops and hands-on activities with a particular focus on basic biotechnology experiments. The nature of the citizen science community raises concerns that its practitioners will not abide by risk assessment and biosafety practices required by law of the professional SynBio community (Schmidt, 2008). The issue is not whether SynBio *can* be safely practiced; it is a question of whether DIY biologists *will* practice it safely. Based on two recent investigative analyses in the USA and Europe, DIY biologists organised in formal member groups will be attentive to biosafety matters (Grushkin *et al*., 2013, Seyfried *et al*., 2014). The SCs note that biosafety laws in European countries, which differ from the USA regulations, require that genetic engineering experiments must be done in GM-certified laboratories, which limits DIYBio. As of mid-2014, only Cork-based DIY biologist Cathal Garvey and the Open bioLab Graz Austria, OLGA, have licenses to carry out genetic engineering experiments, and the number of groups with GM licenses is expected to increase (Seyfried *et al.*, 2014). Though many media reports present DIYbio as the forefront of scientific developments in SynBio, the capacities and capabilities are currently limited.

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

Amateur SynBio and citizen science do not pose any new category of hazard to humans or the environment. While the hazard remains the same, e.g. infection with pathogenic organisms, the probability of unintentional harm might increase, because more people are starting to actively work with biological material. However, as long as the citizen science community is well informed and cautious, the overall additional risk increase would be minimal. Awareness raising, training and keeping up good laboratory practices is a primary duty of the citizen science community, according to their own Code of Ethics. Complementary support by traditional institutional actors will help to achieve the highest training standards.

Question 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?*

Existing methodologies are appropriate for assessing potential risks associated with citizen science. The methodologies must be applied, even outside traditional institutional settings. Several European countries have rules and regulations regarding which authority is supposed to carry out the risk assessment of an experiment scheduled for a

[18]http://diybio.org/codes/

DIYbio laboratory. As long as biological experiments carried out in community labs are transparent and well documented, the application of existing risk assessment methodologies is feasible.

Question 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?*

Risk assessment is facilitated if DIY biologists are/become members of identifiable do-it-yourself groups/community laboratories, where these methodologies can be carried out. Currently, SynBio techniques are difficult and expensive and limit the DIY biologist for the foreseeable future.

3.5 Opportunities for inherent safety

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

There are 4 biosafety levels and safe host strains designed to thrive only under limited laboratory conditions. Safety locks include *in vivo* toxin-antitoxin pairs, auxotrophy, extreme sensitivity to environmental factors such as UV light, conditional origins of replication, insensitivity to phages, etc., and combination systems that consist of multiple safety locks called “gene guard” system (Wright *et al.*, 2013). It is noted that the SCs did not consider this question to refer to risk reduction measures to be taken during use and after release, but to inherent safety measures only. Currently available genetic safeguards, e.g. auxotrophy and kill switches, however, are not reliable enough for most field releases of GMMs and accidental release of contained GMMs, because of the relative high incident of engineered bacteria escaping various genetic safeguard systems due to mutation and positive selection pressure for mutants. However, SynBio might help with an additional safety level that classical GM might not have reached (Wright, Stan, Ellis, 2013). The Gene Guard design is an example of plug-in safety elements. SynBio and, in particular, XB promise strains that have built-in safety locks, i.e. additional layers of biocontainment. There is potential for SynBio to generate organisms that are significantly different from natural organisms to the extent that horizontal gene flow or sexual reproduction with natural organisms would be severely impeded or even made impossible. In combination with well-known engineered auxotrophies, these new strains might represent a significant improvement over current safety locks (Acevedo-Rocha and Budisa, 2011, Budisa, 2014, Marliere, 2009, Schmidt, 2010, Schmidt and deLorenzo, 2012). The same applies to recent work showing additional safety by creating dependency on an amino acid that does not occur in nature (Mandell et al., 2015; Rovner et al. 2015). Although SynBio safety locks, such as the genetic firewall seem promising, no one approach will solve all biosafety risks. A careful evaluation of all possible dimensions (educational, behavioral, technological, economic, etc.) is warranted on a case-by-case basis (Schmidt, 2013).

For the development of protocells, a standardised system of classifying levels of precaution when handling biological agents has been recommended, which uses a scheme similar to the four biosafety levels and that this classification system be developed for working with protocells in the laboratory (Bedau *et al.*, 2009). For risk mitigation by inherent safety mechanisms, the priority is to address the integration of protocells with natural organisms. It is argued that the ephemeral nature of the

protocells allows for a time-limited application of new metabolic features (Lentini *et al*., 2014). This might be relevant for safety and long-term concerns including identified evolutionary uncertainties and has the potential to build a safety lock into newly engineered functions. Although synthetic genomics and DNA synthesis might provide a mechanism by which to incorporate safety locks into SynBio products, e.g., by enhancing the genetic stability of synthetic genomes by removing repetitive and recombination-prone sequences (Annaluru N, 2014), they do not by themselves contribute to the risks or safety of SynBio products.

Building safety locks by the DIY biologist community is not expected, because the development of these locks is beyond the current capabilities of the community. When safety locks become available either generated by the academic or the DIY biologist community, DIY biologists may, however, use them. For example, one of the beneficiaries of a safe host strain might be a DIY biologist community, and would thus allow them to carry out experiments in a safer environment. Other tools such as reliable kill switches might also be of interest to DIY molecular biologists.

3.6 Designing inherently safe applications

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

The definition of SynBio (see Opinion 1, SCENIHR, SCHER, SCCS, 2014) emphasises the facilitation and acceleration of the process including design, which also implies increased predictability. The question arises whether this might mean that all adverse effects for human health and/or the environment associated to SynBio might be avoided by proper design and safety engineering approaches. It is argued that much is learned from safety engineering, e.g. how to design inherently safe systems (Schmidt, 2009). However, it is also emphasised that controlling all biological processes associated with an engineered system is not currently possible. The stochastic and probabilistic character of the underlying biochemical processes limits the drawing of a blueprint. One of the goals of SynBio is to keep up with potential interactions between the engineered system and its environment. The following sections focus on basic safety by design approaches.

3.6.1 Biocontainment - genetic safeguard strategies

In the following section, basic safety by design approaches, as developed by classic genetic engineering, are briefly described.

For physical containment, all modified organisms are kept within the laboratory and are physically separated from the outside world. In addition to physical containment, genetic engineers designed these genetic circuit containment systems for single cell organisms (see figure 3.6.1). The following are examples: 1) Engineered auxotrophic strains are designed to be dependent on a chemical that is not available in nature and that cannot be produced by the strain itself, which generates a strain reliant on the external feeding of the chemical. Escape of this strain from the dependent chemical would lead to death; 2) Induced lethality is a genetic system in an organism in which one gene product silences a second gene product that would be toxic to the cell. If the organism escapes into the environment, spraying a specific biochemical compound that inhibits the first gene product will enable the second, toxic, gene product to kill the organism. In theory, the biochemical compound will only trigger cell death in the engineered cells and will not harm other organisms; 3) Gene flow prevention is another genetic system in which a

toxic peptide is encoded on a plasmid that also contains other information and is inhibited by another gene product encoded on the genome. As long as genome and plasmid occur within the same cell, the toxic system is neutralised by a toxin-antitoxin system. If the plasmid is transferred to another cell by means of horizontal gene transfer, the receiving cell would not be able to inhibit the production of the toxin and will die. These three systems and others, e.g. conditional origin of plasmid replication, are examples of how the spread of genetically engineered cells or its plasmids to the environment could be limited.

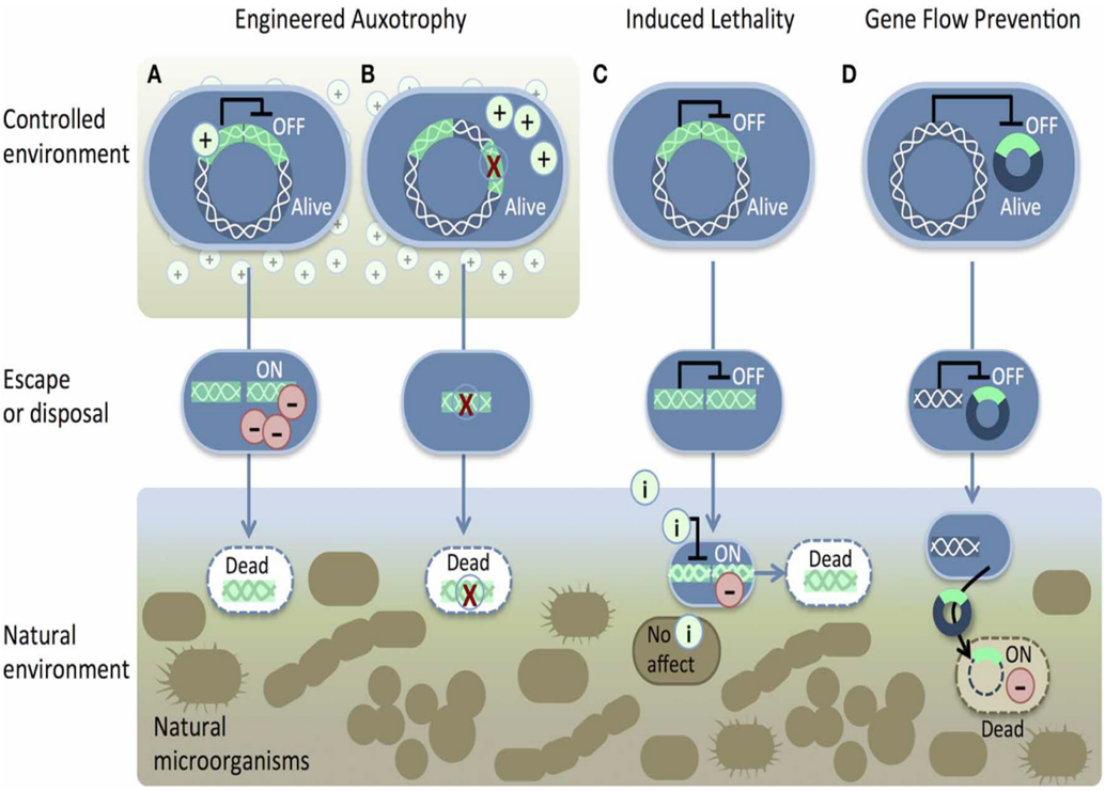


Figure 3.6.1: Schematic overview for three different types of genetic safety locks: (A) and (B) Engineered auxotrophy, (C) induced lethality and (D) gene flow prevention. (Source: Moe-Behrens 2013).

3.6.2 Considerations for using the known containment approaches

Although genetically engineered safeguard systems, e.g. engineered auxotrophic, induced lethality, gene flow prevention offer technical solutions to restrict engineered cells to laboratory or production settings, none function perfectly (Wright *et al.*, 2013).

- Toxin-antitoxin pairs are prone to low but non-negligible rates of failure due to mutations, or their use is limited to certain hosts, i.e. for the other host, the toxin is not toxic.
- Auxotrophy requires that the expression level must be optimised and there is a heterogeneous environment, e.g. soil, water that may remove the selection pressure, because similar or identical substances could be encountered or the cells might be able to regain the metabolic pathway to produce the needed substance themselves. Additionally, induced lethality has limits, because it is unclear if the highly specific toxin would reach all escaped organisms in the required concentration.

- Currently available genetic safeguards, e.g. auxotrophy and kill switches, are not sufficiently reliable for field release because of the relative high incidence of engineered bacteria escaping genetic safeguard systems due to mutation and positive selection pressure for mutants.

Numerical estimates in the literature illustrate the probability of safety system failure. Approximately, one cell in a million escapes the engineered safety mechanism (Schmidt and deLorenzo, 2012, Moe-Behrens *et al*., 2013). A one-millilitre cell suspension with 10^8 cells will contain an average of 100 cells with a failed safety system. Because the failure probability of $1/10^6$ cannot guarantee biocontainment, several strategies are needed. Firstly, a combination of different safety systems in one cell would potentially decrease the probability of containment breach. For example, coupling two independent safety systems with a failure probability of one in 10^6 would lead to an overall failure probability of one in 10^{12} cells. The challenge is to ensure that combined safety systems are independent through orthogonality, which as described in the chapter on xenobiology can be achieved by refactoring codon usage, a genome using an expanded genetic code, or the use of non-canonical biochemistries, e.g., on the level of amino acids and nucleic acids (see table 4).

Table 4. Comparison of different biocontainment strategies in genetic engineering and SynBio

	Auxotrophy	Kill switches	Recoding	Code reassignment	XNA
Stability	Pressure to bypass dependence	Pressure to expel circuits	Dependent on extent of recoding	Pressure to revert	Dependent on if criteria are met
Gene flow	Possible to acquire pathway	Possible	Unlikely	Unlikely but possible to pick up	No
Persistence	Compete with natural microbes	Possible	Possible (susceptibility to phage may be lost)	Unlikely	No
Example	*thyA*	*colEI-ecoRIR*	314 amber codons substituted	UGA as glycine codon	

Source: Krishnakumar 2013 SB6.0, modified

SynBio and orthogonal biological systems are at an early developmental stage, with many scientific and technical questions still unanswered. To generate inherently safe applications, it will be necessary to master orthogonality in biological systems.

3.7 Uncertainty

Characterisation of the uncertainties in a risk assessment of SynBio activities is important for transparency and should also be a valuable aid to risk managers in determining how to respond to risk management advice. In addition, it is a useful way of indicating priorities for further work to improve the robustness of risk assessments. The degree of uncertainty will obviously influence the risk assessment, while the extent to which a quantitative assessment of uncertainty and variability is needed will depend on the context of the risk assessment/risk management as determined in the questions asked i.e. problem formulation. (SCENIHR, 2012).

Analysing uncertainties, a basic element of the Risk Assessment (RA), is an integral part of the assessment process. Uncertainly may be present at all levels of the risk assessment: in identification of the effects, in quantification and modelling of the exposure, and in characterisation of the risks. Essential is an evaluation of the reliability of the assessment as well as the remaining uncertainties and a correct typology of the uncertainties (EC, 2000; EEA, 2001; Van Asselt and Vos, 2005; COMEST, 2005). Thus, the purpose of the uncertainty analysis is to assess the limitations of the information that has an impact on the confidence level in the results of the assessments and, therefore, on the management measures as well as on the research initiatives aimed at filling the most critical data gaps.

The scientific risk evaluation for tools, methods and products of synthetic biology should be based on reliable scientific data and lead to a conclusion on the plausibility, the likelihood and the severity of a hazard's impact. Varying degree of uncertainty exist regarding the predictability of biological properties of partially or completely synthetic agents or materials as well as some unusual potential risks, as "do-it-yourself" (DIY) scientists and others outside of traditional research environments explore the field (Presidential Commission, 2010). In the short term, agents generated through synthetic biology may not raise novel risk assessment or risk management issues. However, as this field is developing fast, risk assessment of more complex, novel, and artificial agents and products of Synthetic Biology will be challenging.

Because of the difficulty of risk analysis in the face of uncertainty—particularly for low-probability, potentially high-impact events in the emerging field of Synthetic Biology—ongoing assessments must be updated as the field progresses.

4 OPINION

This Opinion is the second in a series of three on SynBio responding to questions from the EC. The overall, legal and scientific background underlying these questions from the Commission were discussed in the first Opinion and a definition of SynBio was proposed.

- The SCs have confined the scope of its analysis to the foreseeable future (up to 10 years), acknowledging that its findings should be reviewed and updated again in another decade.
- Outside the scope of the current mandate are the social, governance, ethical, and security implications of SynBio.
- The complex and evolving nature of biological systems means that SynBio will continue for decades to study the basic mechanisms of biology in pursuit of greater control of designed biological systems.
- Recognising that SynBio evolved from and shares much of the methodologies and tools of genetic engineering, it is considered in this Opinion that the risk assessment methodology of contained use activities and activities involving the deliberate release of GMOs are built on principles outlined in the Directives 2001/18/EC and 2009/41/EC and in Guidance notes published in Commission Decision 2000/608/EC.
- Complexity and uncertainty may be confronted within the safety assessment of SynBio. Within the scope of current GMO regulations risk assessment will soon be challenged, e.g. lack of comparator species, increasing number of genetic modifications and engineered organisms, etc.
- Implications have been assessed for 5 research areas and one trend in SynBio: genetic part libraries and methods, protocells, minimal cells and designer chassis, Xenobiology, DNA synthesis and genome editing as well as citizen science.

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

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

I. **Genetic part libraries and methods**: The continued advancement of genetic parts libraries has three implications for human, animal and environmental health and safety. 1) More complete, precise and accurate information on the biological function of parts in genetic libraries will improve the effectiveness of risk assessment. 2) SynBio library construction and parts characterisation may increase the frequency of use of uncharacterised components, and/or the diversity of biological functions used which requires diligent application of established safety procedures. 3) Emergent properties of more extensive genetically engineered systems may present some new challenges in predicting or testing for risks.

II. **Minimal cells and designer chassis**: Minimising the number of components required to support biological synthesis from synthetic DNA circuits or genomes may also simplify control of the function(s). Irrespective of the risk group of the recipient chassis strain, and according to current risk assessment principles of GMM, the assessment of the resulting bioreactor-ready production strain (obtained by the

introduction of different modules) necessitates an evaluation of each element that has been used towards its achievement, thereby including an assessment of the genetic material inserted, the organism and the method used for insertion of the genetic material.

III. **Protocells and artificial cells**: Protocells are non-living vesicles and are likely to be confined to the laboratory for the foreseeable future. The ability for such cells to replicate is the objective, but as explained above is not possible yet. Dispersion is not possible due to lack of cell viability. However, accidental exposure of humans to protocells in the laboratory may occur. As of 2014, protocells present developments that are likely to fall within a regulatory framework covering chemicals rather than within the current GMO regulatory framework. Risks entailed by protocells research today are no higher than the risks in biology and chemistry laboratories (Bedau *et al*., 2009), because current research does not involve the creation of novel, viable artificial cells. Integration of protocells into living organisms and future developments of autonomous protocells warrants the examination of possible routes of exposure and adverse effects. The framework for the risk assessment of such cells should draw on, but not necessarily be confined to, the methodology used for GMO risk assessment. Aspects such as allergenicity, pathogenicity, biological stability etc. should also be considered. Apart from social and ethical implications, this will certainly present challenges to the risk assessment.

IV. **Xenobiology**: The use of non-standard biochemical systems in living cells (e.g. XNA, alternative base pairs, etc.) has two types of implications for risk assessment and biosafety. Firstly, the new variants have to be tested for their risk to human health and the environment and secondly, the xenobiological systems might be engineered to allow for improved biocontainment, i.e. the so-called genetic firewall which aims at avoiding exchange of genetic material through horizontal gene transfer or sexual reproduction between the xenobiological organisms and natural organisms.

V. **DNA synthesis and genome editing**: The new technologies for DNA synthesis and genome editing accelerate the process of genetic modification considerably, and increase the range and number of modifications that are easily possible. In addition to the increased speed of modifications, which might pose risk assessment challenges in itself, the following aspects need special consideration:

- Genetic modifications in higher animals are now possible within a single generation, by direct genome editing of zygotes.
- Many of the new methods allow multiplexed genetic modifications, which affect a large number of loci at the same time. The resulting organisms are screened or selected afterwards, but their risk is not necessarily assessed individually.
- The number of genetic modifications introduced in parallel by large-scale DNA synthesis (large amounts, long-sequences and low costs) and/or highly-parallel genome editing increases the distance between the resulting organism and any natural or previously modified organism to which it could be compared to for risk assessment purposes.

VI. **Citizen science:** In principle, citizen science (DIYbio) does not pose any new hazard to humans and the environment. Because DIYbio is now more popular than in earlier years, increasing the number of participants that could cause harm, it is important

that established safety practices among DIY biologists are maintained. Verifications by an independent biosafety entity should be encouraged. All newcomers must undergo the same biosafety introduction training as individuals in institutional laboratories. Awareness raising, training and keeping up good laboratory practices is a primary duty of the DIYbio community, according to their own Code of Ethics. Complementary support by traditional institutional actors will help to achieve the highest training standards.

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

I. **Genetic part libraries and methods:** The existing methodologies for risk assessment are applicable and appropriate. However, it may be difficult to accurately assess the properties that emerge from interactions of many parts in more complicated systems. Tools to assist in such assessments may be needed.

II. **Minimal cells and designer chassis:** It is possible to use existing methodologies because minimal cells do not raise additional concerns compared to the wild type organisms they are derived from.

III. **Protocells and artificial cells**: Existing appropriate methodologies are available for protocell risk assessment. However, it will be important to select the correct methodologies from the chemical and biological fields, because protocells fall in between chemistry and biology. Therefore, it is crucial to choose the most appropriate combination of methodology for assessing risk.

IV. **Xenobiology**: Existing methodologies are possible to use. However, it is necessary to create and collect data sets and knowledge about the interaction between xenobiological and natural organisms for risk assessors to apply the established methodologies to xenobiological organisms.

V. **DNA synthesis and genome editing**: It is possible to use existing methodologies. The acceleration of the genetic modification process by advances in synthetic genomics and DNA synthesis calls for accelerated procedures for risk assessment, especially where genetic modifications are introduced in a highly parallel manner.

VI. **Citizen science**: The existing methodologies are appropriate. It is essential that the existing methodologies are applied even outside the traditional institutional settings.

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

I. **Genetic part libraries and methods**: Present methodologies are appropriate and adequate to assess the potential risks of activities and products associated genetic parts libraries of SynBio, and to ensure a high level of protection. Nonetheless, the SC suggests that improvements to the methodologies can be made to ensure a continued safety protections proportionate to risk, while also enabling scientific and technological advances to realize the prospective benefits of SynBio. These improvements include: 1) support research and training on tools for predicting emergent properties of genetic systems, 2) streamline and standardise across EU

member states the methods for submitting genetic modification data and genetic parts information to risk assessors, 3) draft brief guidelines for risk assessors on how to evaluate the emergent properties of genetically engineered systems, 4) encourage the use of GMOs with a proven safety record as acceptable comparators for risk assessment. Support 1) research to characterise the function of biological parts, 2) development of computational tools to predict emergent properties of SynBio organisms and their potential failure modes, including principled incorporation of molecular and mechanistic uncertainty in the computational approaches (Breitling *et al*. 2013), and 3) broad dissemination and training in such tools and knowledge resources.

It can be foreseen that the comparator approach for risk assessment will at some point no longer be applicable as SynBio progresses and the resulting systems become ever more different from their natural counterparts. The lifetime of applicability of the comparator approach could be extended by considering non-natural, engineered comparators of demonstrated safe use, in addition to the natural comparators now commonly used.

Moreover, using the technologies described in the sections on genome editing and xenobiology, it is now possible (and may become more prevalent) to make targeted genome modifications, such as introducing novel genetic parts, by only transiently introducing hereditary material produced outside the organism (e.g. by the MAGE and CRISPR techniques), or without ever introducing hereditary material prepared outside of the organism (e.g. using zinc finger proteins). Such modifications would not necessarily be considered genetic modifications according to the current definitions of genetic modification in Directives 2001/18/EC and 2009/41/EC. This might create additional challenges from a risk assessment standpoint, in that organisms produced by these methods may contain more pervasive changes to the genomes of living organisms than traditional genetic modification techniques.

II. **Minimal cells and designer chassis**: No change in the existing methodologies is necessary.

III. **Protocells and artificial cells**: There is a need to establish new combined methodologies addressing both chemical and biological hazards/risks.

IV. **Xenobiology**: It is necessary to create and collect data sets and knowledge about the interaction between xenobiological and natural organisms for risk assessment.

V. **DNA synthesis and genome editing**: The acceleration of the genetic modification process by advances in synthetic genomics (as defined in Opinion I, section 3.3.1.3) and DNA synthesis will challenge the existing case-by-case approach to risk assessment because of the range and higher number of genetic modifications to be assessed. The SCs suggest a focused procedure that identifies and categorises appropriate groups of genetic modifications that can be assessed in a “pooled” manner, thus alleviating the need for individual risk assessment in the case of highly parallel and multiplexed genetic modifications. These pooling protocols will need to take into account that risks might potentially arise from the synergy of combined modifications.

VI. **Citizen science**: The SCs suggest that existing risk assessment methodologies ensuring safe use of SynBio should be applied also outside the traditional institutional settings.

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

The currently available safety locks used in genetic engineering are genetic safeguards such as auxotrophy and kill switches. However, they are not yet sufficiently developed for SynBio. For example, in the case of field releases of GMMs, there is high probability that engineered bacteria may escape various genetic safeguard systems due to mutation and positive selection pressure for mutants. It is possible that SynBio might lead to improved types of containment with much lower probability of failure than classical GM. Examples are 1) the attempts such as the Gene Guard design of plug-in safety elements, 2) SynBio, in particular XB, promises new bacteria strains with built-in safety locks, 3) genetically recoded organisms with reassigned codon triplets, i.e. altered genetic code, and 4) targeted replacement of DNA, base pairs and amino acids throughout the whole organism, with equivalent biochemistry (e.g. XNAs) resulting in constructs with the potential to be significantly different from natural organisms, which would severely impede horizontal gene flow or sexual reproduction with natural organisms. The combination of well-known engineered auxotrophies in these new strains may significantly improve current safety locks. Even though SynBio safety locks, such as the genetic firewall (Schmidt, 2010, Acevedo-Rocha and Budisa, 2011[19]) seem promising, it is important to note that no single technology will solve all biosafety risks (Schmidt, 2013).

For risk mitigation by inherent safety mechanisms in protocells, the priority will be to address the integration of protocells with natural organisms. The ephemeral nature of currently available protocells allows for a time-limited application of new metabolic features, which might be relevant wherever safety and long-term concerns e.g. evolutionary uncertainties, are identified. This approach might have the potential to build safety locks into newly engineered functions.

Synthetic genomics and DNA synthesis might provide a mechanism to incorporate safety locks into SynBio products, e.g. by enhancing the genetic stability of synthetic genomes, by removing repetitive and recombination-prone sequences. However, they do not contribute to the risks or safety of SynBio products directly.

Contributions to built-in safety locks from the DIY (molecular) biologists' community are not expected because the development of these locks are beyond the current capabilities of this community. In the future, however, such developments might become a reality.

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

The definition of SynBio (SCHER, SCENIHR, SCCS, 2014) emphasises the facilitation and acceleration of the process including design, which also implies increased predictability. The question arises whether this could mean that all unintended consequences of SynBio can be avoided by proper design and safety engineering approaches.

[19] http://onlinelibrary.wiley.com/doi/10.1002/anie.201103010/abstract

Controlling all biological processes in an engineered system is a long way to go, if it can be reached at all in view of the stochastic and probabilistic character of the underlying biochemical processes.

General biocontainment approaches are based on physical containment, inhibition of uptake, incorrect translation, inability to replicate, absences of host immunity and endogenous toxicity.

The currently available genetic safeguards, e.g. auxotrophy and kill switches are not reliable enough for a field release, because of the relative high incidence of engineered bacteria escaping various genetic safeguard systems due to mutation and positive selection pressure for mutants.

By adding additional layers of containment through the use of orthogonal systems, new forms of biocontainment seem feasible. A blueprint of a general procedure/strategy for designing inherently safe applications of SynBio thus needs to contain a clear support for the analysis, development, testing and prototyping of applications based on safe and orthogonal biological systems.

The SC agrees that a “blueprint” needs to start with a commitment to actively support the development of inherently safe applications. This commitment has so far been missing, but will be instrumental to keep SynBio in the realm of responsible innovation for the years to come. Further details on research recommendations targeting the establishment of inherently safe applications will be provided as part of Opinion III “Research priorities”.

5 CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this Opinion was opened on the website of the EU scientific committees between 19 December 2014 and 3 February 2015. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

20 organisations and individuals (providing in total 72 comments) participated in the public consultation providing input to different chapters and subchapters of the Opinion. Among the organisations participating in the consultation, there were universities, institutes of public health, industry representatives, NGOs and public authorities.

Each submission was carefully considered by the Scientific Committees and the scientific opinion has been revised to take account of relevant comments. The literature has been accordingly updated with relevant publications.

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_26_en.htm

6 MINORITY OPINION

None

7 ABBREVIATIONS AND GLOSSARY OF TERMS

- Biosafety level (BSL)
- Cartagena Protocol on Biodiversity (CPB)
- Clustered Regularly Interspaced Short 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)
- International Risk Governance Council (IRGC)
- Living Modified Organisms (LMOs)
- Massive Open Online Courses (MOOCs)
- 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)
- New Techniques Working Group (NTWG)
- 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

Questions from the mandate

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

Scope and definition of the phrase "SynBio"

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

Methodological and safety aspects

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

Research priorities

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

9.2 Annex II

ABSTRACT from the Opinion I - Definition

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

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

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

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

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

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

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

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

SynBio includes any activity that aims to modify the genetic material of living organisms as defined in the Cartagena Protocol on Biodiversity. This does not exclude the

consideration of non-viable, non-reproducing goods and materials generated by or through the use of such living genetically modified organisms (GMOs). Genetic Modification (GM) involves the modification of living organisms with heritable material that is independent of the chemical nature of the heritable material and the way in which this heritable material has been manufactured. SynBio uses all available technologies for genetic modification, but in particular, aims at a faster and easier process, which also increases predictability.

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

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

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

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

9.3 Annex III

Application areas

Contained use: the intent is to prevent any interaction between the entity and the natural world, including the effluents of the product or process and accidental releases.

1. Laboratory research

2. Manufacturing, including the production of chemicals and non-living substances

Intentional release: the intent is to release the entity to the natural world including humans, animals, or environment at large

1. Medical, veterinary or cosmetic treatment

    a. Biologics
    b. Vaccines
    c. Topical, physiologically inactive compounds or biologics (e.g., Cosmetics)
    d. Gene therapy including viral delivery agents
    e. Cellular, tissue and organ synthesis/therapies
    f. Human performance enhancement

2. Food, agriculture or food processing (plant, animal and microbial species)

    a. GM plants
    b. GM animals
    c. Food processing technologies (e.g., bacteriophage sterilisation of meat)
    d. Food diagnostics

3. Non-food environmental applications (plant, animal, microbial)

    a. Bioremediation, waste treatment, or mineral extraction
    b. Energy or chemical producing microbes exposed to the environment (e.g., Algal ponds)
    c. Material applications including GM non-food plant & animal products (bioengineered leather, silk, etc.)
    d. Information technologies (environmental biosensors, DNA/Cell/biochemically encoded data)
    e. Engineered leisure species including pets, plants and microbes