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Responsible Research and Innovation in Synthetic Biology

Adaptive Biosafety Assessment as a Learning Process

Strategy Paper Annex 3: working document

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SYN-ENERGENE

Synthetic Biology – Engaging in Responsible Governance of New and Emerging Science and Technology in Responsible Governance of the Science and Society Relationship

Table of Contents

Adaptive Risk Assessment in Synthetic Biology

Working document

This working document was drafted for preparation of a combined expert-stakeholder workshop on Adaptive Biosafety Assessment on June 23, 2016 in Amsterdam. It is a working document in the most literal sense, i.e. a basis for further processing by including results of the workshop and output from other activities on this topic.

1. Introduction

SYNENERGENE *is a four-years mobilization and mutual learning action plan (MMLAP) supported by the European Commission under the 7th Framework Programme. The project aims at initiating and fostering public dialogue on synthetic biology and mutual learning processes among a wide variety of stakeholders from science, industry, civil society, education, art and other fields.*

The issue of biosafety of synthetic biology (synbio) has been discussed on different occasions during the past years. So far, the view of most experts is that existing approaches used in the risk assessment of genetic modification can be applied to experiments in synthetic biology too. At the same time, many experts recognize that the nature of innovative and emerging synbio technologies is uncertain (SCENIHR, 2014). Synthetic biology enables scientists to do experiments with biological systems that differ essentially from naturally occurring ones (Pauwels *et.al.*, 2013), which may no longer be the type of well-known and well-characterized organisms we have been dealing with so far. The Scientific Committees concluded that: "*…complexity and uncertainty are characteristic parts of the risk assessment of Synbio and have led the Scientific Committees 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*." (SCENIHR, 2014).

Several authors of essays and papers focusing on the social and ethical dimensions of synthetic biology have emphasized that this technology triggers similar issues and is / will be perceived as controversial as genetic engineering. The GMO debate has taught us that policies on controversial technologies require governance approaches that include safety as well as normative issues.

This calls for a pro-active attitude in which we anticipate future developments and a transparent, iterative process of risk governance, which includes risk assessment and dialogue

among stakeholders including civil society globally (König *et.al.*, 2013, SANCO, 2012). These are key elements of Responsible Research and Innovation practices which is also a learning process. The challenge is to find ways in which the development of knowledge, expertise and strategies needed for risk assessment keeps pace with developments in synthetic biology research.
In order to develop risk assessment approaches along with new developments in synthetic biology, we have to design a learning process that involves researchers, regulators, risk assessors, stakeholders as well as civil society.

Organized in the context of the SYNENERGENE project, the workshop on June 23, 2016 is a step in this learning process. It will elaborate on previous discussions and documents and focus on four key questions:

1. In which cases may the current approach for GMO risk assessment fall short?
2. What are effective (adaptive) strategies to deal with such cases, maintaining a high level of biosafety without unnecessarily hampering R&D in synthetic biology?
3. What is needed for developing such strategies in terms of knowledge and organization?
4. Which actors should be involved in the process of further developing adaptive strategies?

Before further introducing these key questions, this working document starts with a brief overview of synthetic biology and the key concepts for GMO risk assessment.

2. Synthetic biology

Synthetic biology is usually defined as "the rational design and construction of new biological parts, devices and systems with predictable and reliable functional behaviour that do not exist as such in nature, and the redesign of existing natural biological systems, for basic research and targeted purposes". This kind of definition tells us something about the engineering approach (rational design and redesign, construction) that is applied to biological systems, the goals (reliable and functional behaviour), and the 'naturalness' of the results.

To investigate the need for new approaches to risk assessment we need more detailed description of the type of synbio experiments and applications. In its first report to the European Commission SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCCS (Scientific Committee on Consumer Safety), SCHER (Scientific Committee on Health and Environmental Risks) distinguish 4 research synthetic biology areas (SCENIHR, 2014a):

- Synthetic genomics and DNA synthesis, applied in, for instance, minimized genomes;
- Metabolic engineering, including cell-free *in vitro* systems;
- Orthogonal biosystems / xenobology: xpanding the repertoire beyond the 20 canonical 20

amino acids and developing new biocontainment systems by means of nucleic acid analogues;
- Protocells: chemical vesicles as an initial step towards the synthesis of living organisms.

COGEM and the Scientific Committees[1] identified a number of subfields (COGEM, 2016, SCENIHR, 2014b):

1. Writing synthetic genomes
   Bottom-up synthesis of DNA, genes and complete genomes. This is an enabling technology that allows for new developments in other subfields. The first fully 'synthetic' bacterial genome was announced in 2010, follow by the first synthesized eukaryotic genome (*Saccharomyces cerevisiae*) in 2014. In 2016 plans were announced to synthesize human genomes (Boeke *et.al.*, 2016).

2. Minimal genome research
   Top-down or bottom-up creation of an organism by deleting regions of the genome, which are not essential for cellular life. Minimal genome organisms can be used as a 'chassis ' or production platform to be expanded by genes not present in the parental genome.

3. Metabolic pathway engineering
   Reprogramming metabolic pathways that may involve an unlimited number of genes from various different sources, including synthetic genes. This subfield could be considered an advanced extension of classical recombinant DNA techniques.

4. (Molecular) xenobiology
   Changing the chemical composition of nucleic acids (from DNA to XNA) or production of proteins containing amino acids to do not exist in nature. There are 20 common amino acids, but researchers have identified in the lab over 50 unnatural amino acids that can be incorporated into a peptide.
   The possibility of non-natural alternatives to the natural base-pairs occurring in DNA was already considered in the late 1990's (Eschenmoser, 1999) and functional use *in vivo* has been shown in 2012 (Pinheiro and Holliger, 2012).
5. Synthetic (proto)cells
   Bottom-up constructed cell-like structures built from organic and/or anorganic elements that mimic natural cell components and molecules, capable of sustaining, reproducing and adapting/evolving. Essentially a synthetic cell is a container with a synthetic membrane that

[1] In 2014 the Scientific Committees on Consumer Safety (SCCS), on Health and Environmental Risks (SCHER) and on Emerging and Newly Identified Health Risks (SCENIHR) reported on synthetic biology. This is referred to in the tekst as "the Scientific Committees".

contains a metabolic mechanism to yield and store energy and molecules capable of carrying or transferring information and reacting to the environment.

3. Key concepts in GMO risk assessment

Biosafety covers the range of policies and practices designed to protect workers and the environment (and consumers) from unintentional misapplications or the accidental release/escape of hazardous agents or materials (OECD, 2014). In biosafety assessment the distinction between experiments or production in controlled environments (containment) from which agents or materials may escape unintentionally and intentional releases to the environment is crucial.

In Europe, the safety assessment of GMOs is regulated under Directive 2001/18/EC for deliberate release and Directive 2009/41 for contained use. Both directives have annexes that provide elements to be considered in a risk assessment. Several guidance documents for risk assessment have been provided by the European Food Safety Authority, e.g. on the selection of comparators for the risk assessment of genetically modific plants (2011) and the risk assessment of Genetically modified microorganisms (2006). Most experts believe that current GMO risk assessment methodologies will apply (Pauwels et al., 2013; OECD, 2014, SCENIHR, 2014). Most experts agree that GMO regulations will cover synthetic biology (regarding scope and protocols) and that there is no need to modify current regulation systems (EMBO/EMBL, 2015). Applications such as currently developed protocells or protocell-like systems, which are currently unable to replicate, may be considered chemicals rather than GMOs. In that case GMO regulation and will not apply and such systems will be assessed for potential chemical risks (Pauwels *et.al.*, 2013).

The major key concepts in the GMO risk assessment in the European GMO legislation are (Pauwels *et.al.*, 2013):

- Comparative analysis
  For *contained use*, risk assessors define a containment level for the use of a given GMO, taking into account the level of risk estimated from the combination of the recipient organism, the insert and the vector used for insert transfer. Pathogenicity of the host organism, host range, characteristics of the insert and the vector, biological stability, exposure to humans, severity of the impact and availability of effective therapies are among the parameters used in the assessment.
  For *deliberate release* and/or placing on the market (which in some member states includes gene therapy), a comparative analysis is performed to characterize a given GMO relative to defined non-GM comparator(s) with a history of safe use. Identified differences, for instance in fitness and non-target effects, are further evaluated taking into account the range of

natural variation. Parameters used in the assessment are, among others, persistence and invasiveness, gene transfer, interaction with target and non-target organisms and human and animal health.

- Case-by-case approach
  The risk assessment has to be carried out on a case-by-case basis. The required information may vary depending on the type of the GMOs concerned, their intended use and the potential receiving environment, taking into account, i.e., GMOs already in the environment.
- Step-by-step principle
  The containment of a given GMO is reduced and the scale of release increased gradually as the knowledge about the behaviour and impact of the GMO generated by the experiments increases and provides proof that the risk of the next step is negligibly small.

4. New challenges in predicting risks

The Scientific Committees conclude that "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." (SCENIHR, 2014). However, the complexity of emergent properties and the lack of suitable comparators may also necessitate new approaches in risk assessment. They identified four cases:

1. Routes of exposure and adverse effects arising from the integration of protocells into living organisms and future developments of autonomous protocells. Potocells can be engineered to interact with living cells and enhance overall system functionality. If autonomous protocells capable of growing, reproducing and evolving are created in the future, the genetic information that controls internal functioning may mutate. A population of protocells with different genetic information could undergo selection and new photocells could arise. Such applications may need combinations of chemical and biological assessments.
2. New xenobiological variants and their risk on human health and the environment that should be engineered for improved bio containment.
3. DNA synthesis and direct genome editing of zygotes, which enables modification in higher animals with a single generation.
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.

The Scientific Committees also discussed the implications of the rapidly evolving Do-It-Yourself biology citizen science community, which may increase the probability of unintentional harm.

5. Effective (adaptive) risk assessment strategies

This working document provides a non-limitative number of options for risk assessment and management strategies that have been proposed and discussed in various publications.

1. Applying the highest safety level
   In case of high level of uncertainty (many unknowns regarding the nature of the recipient organism, lack of comparator(s), high complexity of traits) the step-by-step principle could be translated in applying the highest safety level to experiments. (Pauwels *et.al.*, 2013).

2. Regular monitoring
   In 2008 COGEM concluded that it is impossible to predict all future developments, which makes it practically impossible to make any judgements about the suitability of the risk analysis in the long term. Therefore, it is important to recognize potential risks at an early stage, which can be done by regular monitoring and sharing information on the results of research and development flows freely between the various government departments and agencies (COGEM, 2008). This option also includes well-designed post release monitoring plans (Pauwels *et.al.*, 2013).

3. Predictive mathematical models
   One of the disciplines involved in synthetic biology is mathematical modelling, where it serves as a (*in silico*) tool for an engineer to predict how a network will behave when it is modified in certain ways (Chandran *et.al.*, 2008). It serves as a crucial link between the concept and realization of a biological circuit. Systems biology and modularity in cellular systems plays an important role in developing such models. Developing such mathematical models is usually an iterative process, in which the results of *in vitro* or *in vivo* tests are used for fine-tuning the model (Kitney and Freemont, 2012). In principle, such models can also be used in risk assessment.

4. Establishing "omics" profiles
   Genomics, proteomics and metabolomics studies may provide relevant data for performing a thorough risk assessment. This approach has recently been tested in GRACE, a EU program for testing food safety assessment approaches for GM maize (Kok, 2012).

5. Designing inherently safe applications

Apart from the type of physical containment measures currently used when working with GMOs biological containment strategies can be applied, such as inability to replicate or endogenous toxicity, which would make engineered organisms more or less safe for use. The European Commission asked the Scientific Committees to draw a blue print of a general procedure/strategy for designing inherently safe applications of synbio.

The Scientific Committees answered that such a blue print is demanding because of the stochastic and probabilistic character of the underlying synbio processes. *Currently available 'safety locks' based on auxotrophy*[2] *and kill switches*[3] *are not yet sufficiently reliable for field releases of engineered bacteria because of mutations and positive selection pressure for mutants that may lead them to escape safeguards* (*SCENIHR, 2014;* Schmidt and De Lorenzo, 2016).

Another possibility is xenobiological approaches based on dependency on xenobiotic amino acids and nucleotides or the use of alternative genetic codes.

6. Developing safe GMOs or chassis

   The report of the Scientific Committees recommends encouragement of the use of GMOs with a proven safety record as acceptable comparators for risk assessment (SCENIHR, 2014). Chassis-organisms usually are / will be generated from non-pathogenic organisms or organisms with a negligible low pathogenicity. Moreover, most of these organisms are expected to be auxotrophs (see next bullet) and thus unlikely to propagate outside defined laboratory conditions (Pauwels *et.al*., 2013).

7. Extended safety training

   Because of the interdisciplinary nature of synthetic biology more stakeholders, like engineers, need to be aware and trained in biosafety issues (EMBO/EMBL, 2015).

8. Applying governance approaches

   The need to link the scientific debate on synbio risk assessment to wider governance approaches is mentioned most papers and documents. Governance refers to the process through which rules, norms and actions are produced, sustained, regulated and held accountable. Risk governance deals with the identification, assessment, management and communication of risks in a broad context. It includes the totality of actors, rules, conventions, processed and mechanisms and is concerned with how relevant risk

[2] Auxotrophy is the inability of an organism to synthesize a particular organic compound. For example by inserting the *URA3* gene, which encodes orotidine-5'-phosphate decarboxylase, an essential enzyme in pyrimidine biosynthesis in *Saccharomyces cerevisiae (Pronk, 2002).*

[3] Kill switches are conditional killing systems by applying lethal genes and regulatory elements.

information is collected, analysed and communicated, and how management decisions are taken. It applies the principle 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 politically acceptable (IRGC, 2008).

Apart from analysis of direct costs and benefits of specific synbio applications it could / should include analysis of the costs and benefits of alternatives as well as the trade-offs between risks and benefits (who gains, who bears the risks) (EMBO/EMBL, 2015).

At present, similar but more pro-active principles have shifted a step further upstream in the research and innovation process. Responsible Research and Innovation (RRI) is a concept already promoted in European science and technology policy making and is defined as "A transparent, iterative 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)" (Von Schomberg, 2011).

6. Needs

During joint workshops by the MIT Program on Emerging Technologies and the Wilson Center's Synthetic Biology Project in 2014 participants identified a number of areas as hurdles to understand the potential ecological effects associated with the release of organisms modified using synbio (Drinkwater *et.al.*, 2014). We mention just a few examples here:

1. Comparators

   Alternative testing schemes in the absence of present-day analogues, possible even in "no analog" environments. This requires more knowledge about causal networks (cause and effect relationships): what they are, what they are connected to, and how their effects may differ based on surrounding environments. It also requires a systematic process for making comparisons and establishing an environmental baseline (Snow *et.al.*, 2005).

2. Identification and prioritization of traits of concern (also mentioned in the Scientific Committees report)

   - More should be done to understand which phenotypes are most relevant to ecological consequences over the short and long term,
   - more emphasis should be placed on understanding the function of a trait, as opposed to fixating on the origin of its DNA (also: Engelhard, 2016), and

- the degree to which context affects the characteristics of a phenotype must be better understood and characterized.

3. Learning from endosymbionts

   Endosymbionts are organisms that live within the body or cells of other organisms. Their parasytic or symbiontic lifestiles are programmed thorugh reduced genomes when compared to their closest free-living relatives. They may therefore offer fundamental insights into the process of genome minimisation and how the process of minimisation itself influences risks (SCENIHR, 2014).

4. Combining frameworks for chemical and biological risk assessment

   If protocell research progresses towards autonomous, replicating chemical systems, which interact dynamically to changes in their environment, a case-by-case approach drawing upon a combination of regulatory frameworks for GMO and chemicals and drugs will be required.

5. Developing environmental models

   Identify metrics needed for measuring fitness, genetic stability, and lateral gene transfer and interactions in synbio organisms with consistency, reliability and confidence.

6. Identify degrees of biological and physical control

   Organisms designed to survive and deliver their designed traits and functionalities at various intervals depending on the application, such as pollutant degraders that have to evolve in concert with surrounding environmental changes, require adaptive evaluation schemes in order to capture varying designs in relation to the organism's engineered purpose and function.

7. Setting up systems for monitoring and surveillance

   Identify basic tools that provide advanced surveillance capacities, e.g. metagenomics for conducting baseline surveys, develop a "barcoding" system. If the assessment target is uncertain or the application is designed to evolve and adapt over time, additional abilities may be needed.

8. Modeling (also mentioned in the Scientific Committees report)

   What modelling tools exist for synbio organisms and are they sufficient for situations where organisms produced using synbio are released into the environment? Can existing models be combined across disciplines, or are new approaches needed to integrate natural, physical, and social sciences with engineering?

9. Standardization of methods and data

What is needed in order to standardize testing methods, data reporting, and organism characterization for ecological evaluations? How to handle data collection and integration and who is responsible for developing, promoting and enforcing standards?

10. Clear strategy for using orthogonal systems[4]

    The Scientific Committees recommend a clear strategy for the analysis, development and testing and prototyping of applications based on new forms of biocontainment and additional layers of containment using such orthogonal systems (SCENIHR, 2014).

11. Standardization of data submitted to risk assessors

    The Scientific Committees also recommend streamlining and standardization of methods for submitting genetic modification data and genetic parts information to risk assessors (SCENIHR, 2014).

12. Adaptive risk assessment methods

    Risk assessment methods should advance in parallel with synbio advances (SCENIHR, 2014).

13. Sharing data and global access to data

    Adaptive risk assessment strategies are flexible and include the latest insights regarding the elements mentioned above. This can be facilitated by easily accessible synbio monitoring data, models and so on, which requires this to be organized at a global level. The Scientific Committees report recommends support of sharing information about specific parts, devices and systems with risk assessors.

14. Effective risk governance and RRI practices

    How to effectively engage with society including various stakeholders: industry, research, civil society, policy? How to initiate an attitude of mutual responsiveness? (What) can be learned from governance practices applied in other technologies, such as nanotechnology?

    There is no blueprint for effective governance practices. Nonetheless, several tools are developed for applying Responsible Research and Innovation principles to new and emerging technologies[5]. These tools still need fine-tuning for application in specific situations and there is still a need to define the concept of the responsibility in different context (social, political, economical) and what it means for different stakeholders.

[4] Biological systems whose basic structures are so dissimilar to those occurring in nature that they can only interact with them to a very limited extent, if at all.

[5] http://www.rri-tools.eu/project-description

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