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ANNOUNCEMENTS AND REPORTS

Event report: SynBio Workshop (Paris 2012) – Risk assessment challenges of Synthetic Biology

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Abstract In Europe and beyond, several advisory bodies have been monitoring the developments in the field of Synthetic Biology. Reports have been sent to national governments for information on the developments and possible regulatory and risk assessment questions raised by this field. To put the issues in a broader perspective, four national biosafety advisory bodies (the French High Council for Biotechnology, the German Central Committee on Biological Safety, the Netherlands Commission on Genetic Modification and the Belgian Scientific Institute of Public Health (Biosafety and Biotechnology Unit)) decided to join forces and organize an international scientific workshop to review some of the latest scientific insights and look into possible challenges in the risk assessment of Synthetic Biology. The *SynBio Workshop (Paris 2012) – Risk assessment challenges of Synthetic Biology* took place on the 12th of December 2012 and gathered scientists from biosafety advisory bodies from fifteen European countries, from the European Food Safety Authority as well as representatives of the European Commission, together with research scientists selected for their excellence in the field. The workshop was divided into two sessions: the first session gave an overview of four major fields in Synthetic Biology. The second session was set up for discussion with a scientific panel and the audience to identify and address relevant questions for risk assessment raised by recent and future developments of Synthetic Biology. An overview of the workshop and the discussion points put forward during the day are discussed in this document.

Keywords Synthetic Biology · Risk assessment · Biosafety · GMOs · New techniques · Emerging risks

The views or positions expressed in this event report do not necessarily represent the official opinion of any of the advisory bodies that initiated the SynBio Workshop (the French High Council for Biotechnology (HCB); the German Central Committee on Biological Safety (ZKBS); the Belgian Scientific Institute of Public Health (WIV-ISP, Biosafety and Biotechnology Unit (SBB)) and the Netherlands Commission on Genetic Modification (COGEM)). The advisory bodies assume no responsibility or liability for any errors or inaccuracies that may appear in this event report.

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1 Introduction

Synthetic Biology (SB) is a rapidly evolving field combining different disciplines that go beyond biology, including engineering, chemistry, physics, computer science and bioinformatics. It can be described 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. Major SB approaches consist of engineering nucleic acid-based biological circuits, defining minimal genomes and/or minimal living organisms, constructing protocells, synthetic genomes and/or synthetic cells. SB also includes a novel approach based on the development of orthogonal biological systems, in which the genetic information is encoded by different chemical structures (xenobiology).

Most current developments in SB involve genetic modification. In Europe, products of genetic modification techniques (genetically modified organisms or GMOs) are specifically regulated under Directive 2001/18/EC for deliberate release and Directive 2009/41/EC for contained use and are submitted to defined risk assessment procedures. The regulatory definitions of GMO and some of the key concepts in the GMO risk assessment relevant to this report are outlined in Fig. 1.

Taking into account the current GMO risk assessment methodologies, it is likely that sufficient information will be available to assess the potential risks for human health and the environment associated with SB products developed using well-characterised organisms and genetic material. It is also expected that in the short term, activities in SB will focus on research and development or commercial production of substances in contained facilities. However, it should be emphasized that SB offers the perspective to develop organisms that could differ fundamentally from naturally occurring ones, hence potentially raising specific issues or challenges as regards the risk assessment principles and methodologies currently applied to evaluate GMOs.

For these reasons, research and developments in SB have been closely followed by risk assessors of GMOs. The field of SB is expanding rapidly and could raise challenges as regards the identification of appropriate comparators (well-characterized organisms with a given risk potential, which are used for risk assessment of yet-uncharacterized organisms by comparing their properties), gathering of relevant information allowing characterization of the potential hazards and/or prediction of the behaviour of such engineered organisms in case of intended or unintended release into the environment.

Genetically modified organism (GMO)
"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" (Directive 2001/18/EC). In this Directive, **organism** means "any biological entity capable of replication or of transferring genetic material".

Genetically modified micro-organism (GMM)
"Micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination" (Directive 2009/41/EC). In this Directive, **micro-organism** means "any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, and animal and plant cells in culture".

Genetic modification
Directive 2001/18/EC (deliberate release) and Directive 2009/41/EC (contained use) include annexes that give additional information regarding the techniques i) that result in genetic modification, ii) that are not considered to result in genetic modification or iii) that result in genetic modification but yield organisms that are excluded from the scope of the Directives.

Comparative analysis
For deliberate release and/or placing on the market, a comparative analysis is performed to characterize a given GMO relative to defined non-GM comparator(s) with a history of safe use. For example, the current risk assessment strategy for GM plants and derived food and feed includes a comparative analysis of the compositional, agronomic and phenotypic characteristics of the GM plant and its appropriate comparator(s). Identified differences are further evaluated taking into account the range of natural variation.
For contained use, risk assessors define a containment level required 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.

Case-by-case approach
Potential adverse effects on human health and the environment, which may occur directly or indirectly, should be assessed on a case-by-case basis. This assessment shall be conducted taking into account the scale of the activities carried out with the GMO (e.g. the nature of manipulation during containment, the environmental impact) and the specificities of the receiving environment.

Step-by-step principle
The introduction of GMOs into the environment should be carried out according to the step-by-step principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken.

Fig. 1 Definitions and key concepts for GMO risk assessment in the European legislation

In 2012, four EU biosafety advisory bodies (the High Council for Biotechnology (HCB, France), the

Central Committee on Biological Safety (ZKBS, Germany), the Netherlands Commission on Genetic Modification (COGEM, The Netherlands) and the Scientific Institute of Public Health, Biosafety and Biotechnology Unit (WIV-ISP, SBB, Belgium)) have shared views on whether the principles and methodologies currently enforced for GMO risk assessment could be challenged when applied on organisms or products developed by means of SB. It was concluded that it would be beneficial to gather expertise at large by inviting risk assessors and research scientists to a one-day scientific workshop on the risk assessment challenges of SB.

This report gives an overview of the main issues and discussion points put forward during the workshop. Although other aspects such as self-regulation, biosecurity, public engagement, governance and ethics also need further consideration in view of an effective regulatory oversight of SB, it is important to note that the workshop was specifically designed to tackle risk assessment issues.

2 Scope of the workshop/methodology

Most subfields of SB are rooted in genetic modification, for which a comprehensive regulatory frame has been developed in the past two decades. While different initiatives and programmes have dealt with diverse biosafety aspects of SB, no coordinated initiative of advisory committees for biosafety has taken place up to now. These advisory committees have a central role in GMO risk assessment in Europe and deal first-hand with risk assessment of products derived from SB research and development, which might prove to be more challenging than the risk assessment of current gene technology products due to their possible novelty and/or complexity.

Having pursued their SB-related activities separately before, the four advisory bodies brought together risk assessors, researchers and regulators from European advisory committees on biosafety, the European Food Safety Authority (EFSA) and the European Commission to exchange on how procedural elements, general principles and/or criteria pertaining to risk assessment might be challenged by the fast-paced progress in SB. Thus, this one-day workshop created an opportunity for coordination and dialogue at the European level. Speakers, scientific panel and steering committee members are detailed in Fig. 2.

After an introductory talk, which reminded the principles and methodologies currently enforced for GMO risk assessment and potential challenges posed by SB development, the first part of the programme was dedicated to the current scientific developments in four major fields of SB (metabolic pathway engineering, synthetic genomics, protocells and xenobiology). Distinguished speakers were invited to give an overview of their fields, highlight recent developments and share their views on possible biosafety issues (Fig. 2).

In the second part, a panel of experts with significant expertise in risk assessment, risk management and/or research was gathered to discuss with the audience a set of questions articulated by the workshop steering committee. This discussion was moderated by Prof. Herbert Pfister, the chairman of the ZKBS. The aim of the discussion was to address elementary questions across the different subfields of SB, i.e. (i) which developments could possibly challenge the comparative approach and/or the case-by-case approach, (ii) which data would be particularly critical or challenging for performing a proper risk assessment, (iii) how uncertainty should be dealt with in contained use and in deliberate release, and (iv) whether and how SB should be dealt with in the current GMO regulatory framework.

The closing session was designed to highlight different considerations and consensus reached in the discussion.

3 Scientific developments in Synthetic Biology

This section provides an overview of the oral communications given during the workshop on the scientific developments and possible biosafety issues in four different subfields of SB: metabolic pathway engineering, protocells, synthetic genomics and xenobiology.

3.1 Metabolic pathway engineering

Prof. Jean-Loup Faulon (University of Evry/Institute of Systems & Synthetic Biology, Genopole, France) introduced the field of metabolic pathway engineering from a historical perspective of metabolic engineering achieved through strain selection or direct pathway modification. As these techniques have been used for many years, he did not consider metabolic pathway engineering as a typical subfield of SB. Besides, while metabolic engineering aims at the bioproduction of chemicals, SB has the broader goal of engineering biological components and systems that do not exist in nature.

Speakers

Katia Pauwels (SBB, Belgium): Introductory talk
Jean-Loup Faulon (University of Evry, France): Metabolic pathway engineering
Steffen Mueller (Stony Brook University, United States): Synthetic genomics
Stephen Mann (University of Bristol, United Kingdom): Protocells
Nediljko Budisa (Technical University Berlin, Germany): Xenobiology

Scientific Panel

Alain Blanchard (INRA, France)
Pascal Boireau (Anses - HCB, France)
Dominique Buzoni-Gatel (INRA, France)
Marc Eloit (Institut Pasteur, France)
Lei Pei (International Dialogue and Conflict Management, Austria)
Alfred Pühler (Bielefeld University, Germany)
Joyce Tait (Innogen, United Kingdom)
Simon Warne (Health and Safety Executive, United Kingdom)

Steering committee

Philippe Herman (Head of SBB)
Didier Breyer (Scientific officer, SBB)
Katia Pauwels (Scientific officer, SBB)

Herbert Pfister (Chairman of the ZKBS)
Birgit Schönig (Scientific officer, Office of the ZKBS)
Marion Kaspari (Scientific officer, Office of the ZKBS)

Frank van der Wilk (Executive Director COGEM)
Ruth Mampuys (Coordinator Subcommittee Ethics and Societal Aspects, COGEM)

Jean-Christophe Pagès (President of the Scientific Committee, HCB)
Catherine Golstein (Scientific and European Affairs officer, HCB)
Marion Pillot (Scientific officer, HCB)

Fig. 2 Speakers, scientific panel and steering committee members of the SynBio Workshop (Paris 2012)

Metabolic engineering is a discipline aiming at engineering cell factories for the bioproduction of chemical and pharmaceutical products (Stephanopoulos 2012). According to Prof. Faulon, this field is moving forward from the improvement of the production of native metabolites to the production of nearly any desired (bio-)chemical, pharma- and neutraceutical component. The field of SB is providing useful tools to metabolic engineering for building non-natural pathways through synthetic DNA constructs that are difficult to produce using traditional genetic engineering techniques. Although the actual size of the "chemical space" (i.e. the number of chemicals that can be accessed by metabolic engineering) is not known, it is believed that SB can greatly enhance the number of components that can be produced in engineered microorganisms. Furthermore, modular constructions of genetic devices such as advanced molecular switches can be used for the combinatorial optimization of metabolic pathways.

Continuing, Prof. Faulon distinguished three types of engineering:

- Natural heterologous: using an insert or pathway from one donor organism in a host organism.
- Non-natural: using a pathway with different parts from different donor organisms.
- New chemistry: evolving enzymes allowing new reactions with slightly different products and inserting genes coding for these enzymes into a host organism (Curran and Alper 2012).

In his presentation, attention was also given to the increasing role of computational tools, as in retrosynthesis (Carbonell et al. 2011; Planson et al. 2012), and experimental approaches in the design process of heterologous chemicals.

Prof. Faulon ended his presentation by concluding that metabolic engineering can greatly benefit from developments made in SB, and in particular for the synthesis and control of non-natural biochemical pathways. SB can too benefit from the methods of metabolic engineering in the areas of pathway analysis, optimization and design (Zhang et al. 2012). He stressed as crucial the future availability of more genetic codes for different compounds, more model or chassis strains, improvements in synthesis efficiency and the streamlining of design and production processes.

3.2 Protocell models as a step towards synthetic cellularity

Prof. Stephen Mann (University of Bristol, United Kingdom) reviewed recent approaches involving the use of protocell models as a step towards the design and construction of synthetic cellularity. Most of the presented work dealt with the generation of compartmentalized chemical reactions. At this moment the so-called protocells could be viewed as sophisticated nano-bioreactors. These approaches also aim at providing elements in the future to achieve the transition from nonliving to living matter. He explained how a continuum might be seen from chemical origins onwards to protocells (basic autonomy), minimal life and finally Biology (life as we know). Whereas efforts towards the construction of minimal cells have been driven by simplification of

biological cells, the study and use of protocell models focus on the design and (re)constitution of cellular functions and synthetic cellularity by compartmentalization (Dzieciol and Mann 2012; Mann 2012).

Achieving compartmentalization is a key requirement for engineering protocells. It is sought by different self-organisation means, as shown by the three following examples:

- Lipid-based vesicles, where various components are trapped inside organic bilayer vesicles (e.g. phospholipids or fatty acids) to circumvent the problem of impermeability (Stano et al. 2011),
- Inorganic nanoparticle based membrane vesicles (silica nanoparticles that contain pores of 20 nm),
- Membrane-free peptide/nucleotide droplet formation by phase separation that enables the diffusion of molecules.

Several examples of gene-free systems were also discussed as part of protocell research, including:

- The reconstitution of cellular functions such as cytoskeletal formation in vesicles of phospholipids and membrane proteins,
- The enzyme catalysis in bioinorganic compartments or membrane-free droplets enabling for instance the production of a compound inside the protocell-like system upon the addition of a substrate in the outside medium,
- The enzyme-mediated nucleic acid synthesis in bilayer vesicle membrane allowing for RNA replication, transcription and PCR (Polymerase Chain Reaction).

In addition, recent studies with cell-free gene expression in synthetic vesicles were presented. These systems have the advantage of using purified recombinant compounds: the mere addition of DNA or RNA molecules to the system is sufficient to generate the desired corresponding products.

At the end of his talk, Prof. Mann concluded that protocells are most interesting bioreactor models for conducting basic research. He also emphasized that the current protocell-like systems have no evolutionary capacities and that developments in this field are still far away from constructing artificial life with autopoiesis properties (Stano and Luisi 2010; Noireaux et al. 2011).

3.3 Promise and peril of synthetic genomics

According to Dr. Steffen Mueller (Stony Brook University, NY, USA), the continuing improvements in DNA synthesis technology hold serious potential to transform the biological sciences. De novo gene and genome synthesis liberates the investigator from the restrictions of the pre-existing template and allows for the rational design of any conceivable new nucleotide sequence. In his presentation, he emphasized that the status of synthetic genomics is not the result of a single transformative technology but rather a result of incremental improvements of many techniques, methods and tools that have been used in molecular biology and genetic engineering for over 30 years.

In his presentation, Dr. Mueller first gave an overview of different synthesis methods and discussed the challenges and limitations of the current state of the art of synthetic genomics. The oligonucleotide synthesis costs have dropped and the price gap between oligonucleotide-reconstructed genes and cloned genes is still narrowing. Unfortunately, it is not possible to synthesize long pieces of DNA without errors (Carr et al. 2004). The correct assembly of oligonucleotides is a limiting factor that will become more and more critical as SB projects become more complex. Dr. Mueller stated that at the moment there are no promising techniques on the horizon regarding the improvement of the accuracy of oligonucleotide synthesis. This is also the case for the developments of methods without the need for synthetic oligonucleotides.

Two milestones of synthetic genomics were discussed from their scientific and societal/political perspectives: the synthesis of poliovirus by Stony Brook University (Cello et al. 2002) and the synthesis of a full bacterial genome (*Mycoplasma mycoides*) by the J. Craig Venter Institute (Gibson et al. 2010). Besides the achievements in the synthesis of whole genomes, the role of synthetic genomics in large-scale mutagenesis was further explored based on the example of Synthetic Attenuated Virus Engineering (SAVE) and its role in vaccine development (Coleman et al. 2008; Mueller et al. 2010).

SAVE is based on codon deoptimization, leading to attenuated viruses that can be used as vaccine candidates. Several codons are known to encode the same amino acid. However, in a defined organism, some codons and tRNAs occur more often than others (they are more 'optimal'). The statistical frequency at which a specific codon pair is used can be predicted. Viruses tend to follow the coding biases of their host genomes. Consequently, by using a computer algorithm, the genetic code of the virus can be redesigned into a deoptimized state, leading to 100 % identity on protein level, but being significantly different on nucleotide level. The resulting viruses are

far less efficient regarding viral translation and replication. In turn, this inefficiency increases the possibilities of the cell to detect and respond to the viral invader. Similarly, released viral loads are low and thus more susceptible to adaptive immunity. Theoretically, natural selection processes may lead to some parts of the virus evolving back into their old 'optimized' state over time, but because the attenuation is based on many hundreds of nucleotide changes, reversion to its wild type virulent form is highly unlikely ("death by a thousand cuts").

Dr. Mueller concluded his presentation by addressing other challenges and concerns in the field of synthetic genomics. A frequently expressed concern is the potential of dual use and the creation of human pathogens (Carlson 2003). Dr. Mueller questioned our collective capacity to regulate the synthesis of oligonucleotides because it will become increasingly difficult to oversee as the components are easily available and accessible to everyone. It would thus make more sense to focus on preparing to face a threat rather than to focus on prevention. A list of viruses really constituting a bioterrorist threat should be defined and vaccines against them should be developed. The chemical synthesis of poliovirus was a wake-up call that viruses can never be considered extinct. Consequently, ending specific vaccination programmes for existing viruses might also make viruses more interesting as bioterrorist agents. Rather than the possibility of a completely new pathogen that is unlike anything we have seen thus far, according to Dr. Mueller, it is more probable that something will emerge that we are already familiar with, and thus can be prepared for.

From a broader perspective, Dr. Mueller pointed out that there is no unambiguous definition of SB and that several technologies that now fall under its scope have been used for many years. The term "Synthetic Biology" should perhaps best be regarded as an accumulation of tools rather than as a new discipline in itself. It is a logical continuum emerging out of the more traditional realms of recombinant DNA technology.

3.4 Xenobiology

Prof. Ned Budisa (Berlin Institute of Technology/TU, Berlin, Germany) first introduced the audience to different concepts of life, from Gottfried Wilhelm Leibniz and Erwin Schrödinger to Tibor Ganti and John von Neumann. He presented the central dogma of molecular biology, which postulates that genetic information flows from DNA transcribed into RNA, which in turn is translated into proteins, and relies on the genetic code universally used on Earth. This genetic code builds on the four nucleotides guanine, adenine, thymine and cytosine. This explains how all cells share a common set of chemistry, macromolecules, information processing and organization of metabolic pathways.

He then explained that artificial life could be created either by the "bottom-up" approach (creation of life from non-living matter) or the "top-down" approach (reduction of pre-existing life forms with the possible subsequent introduction of novel traits). The bottom-up approach was discussed as early as 1911, when Jacques Loeb formulated the goal that the creation of artificial living beings would once succeed, and if not, that the reasons for this impossibility should be found out. The top-down approach has been followed since the 1970s, when classical gene technology was introduced.

According to Prof. Budisa, SB and xenobiology differ in that SB assembles novel living systems by combining interchangeable parts from natural biological systems (creating GMOs) whereas xenobiology uses non-natural (xeno-)molecules for the production of novel biological characteristics and systems, creating Chemically Modified Organisms (CMOs). Amongst other techniques, the CMOs using new nucleotides can be created by applying a direct evolutionary pressure to cells of choice (Marlière et al. 2011).

Finally, he presented options to engineer and expand the genetic code in order to be able to engineer proteins consisting of non-canonical amino acids or even to adapt entire proteomes. This can either be achieved by engineering components by reprogramming the flexibility and tolerance of cellular systems or by orthogonalization (introducing non-interacting aminocyl tRNA-synthetase:tRNA pairs or metabolic pathways without cross-reactivity with the native metabolism). According to Prof Budisa, xenobiology offers the opportunity to generate a "genetic firewall" as a biosafety tool as organisms with heritable material based on non-canonical nucleic acids would not be able to exchange genetic material through horizontal gene transfer or sexual reproduction (Acevedo-Rocha and Budisa 2011; Schmidt 2010; Marlière 2009). Nevertheless, these so-called xeno-organisms could interact and compete for resources within the environment. He hypothesized that any escape of a xeno-organism from direct human control would automatically lead to the death of that organism, as it would be totally dependent on external supply of essential

biochemical building blocks. He finished with emphasizing that the scientific development of xenobiology is in an early phase and has been restricted to contained use so far.

4 Discussion

Following the oral presentations, the audience was invited to exchange views with the panel members on whether and how procedural elements, general principles and/or criteria in the current GMO risk assessment methodology could be challenged when applied on organisms/products developed by means of SB.

4.1 Data relevant for risk assessment

The identification and gathering of data relevant for a comprehensive risk assessment raised several questions. For SB applications, some participants underlined that information gathered according to the current risk assessment approach for GMOs was sufficient. On the other hand, some discussion points also illustrated potential challenges for the risk assessment of organisms generated by SB. As SB encompasses different approaches and techniques, the level of data requirement for the assessment of resulting organisms was found to differ accordingly.

In cases where synthetic biological parts are assembled to enable "metabolic pathway engineering", the approach could be considered as an advanced extension of classical recombinant DNA techniques. However, the SB approach offers the potential to build whole systems using an unlimited number of traits derived from different donor organisms. Even if the sources of all parts of a synthetic organism are known and every new genetic circuit understood, it could be difficult to assess the interactions between all of these parts or circuits and to predict whether the organism would have any unexpected emergent properties. For example, one of the participants pointed out that many metabolites have a signalling function in distinct metabolic pathways, underscoring the need to ensure that pleiotropic effects are properly assessed in terms of their outcome and potential risk to human health and the environment. Therefore, the higher order of combination and complexity could make risk assessment more difficult.

Within this regard it was considered whether the qualitative approach of performing risk assessment should gradually be complemented by a quantitative approach. The risk assessment of GMOs is currently mainly based on a qualitative approach, involving a weight-of-evidence approach and using qualitative estimates to formulate the level of risk (high, moderate, low or negligible). A more quantitative approach could be of particular relevance for organisms with a higher order of combination of parts and an increased number of new interactions to be assessed. Risk assessment could benefit from computational aids in order to improve the quantitative approach. However, it would also necessitate gathering relevant data to build appropriate baseline information, i.e. information related to natural comparators. This would be necessary to fulfil one of the current principles of the GMO risk assessment methodology: the comparative approach (Fig. 1). This comparative approach could be particularly challenged in cases where molecules not known to be present in nature are produced. For those cases where an appropriate comparator will be lacking, a comprehensive safety assessment will be necessary taking into account the scope of the use of the organisms (contained use versus deliberate release).

"Omics" technologies have been proposed as one possibility to generate data useful for risk assessment of GMOs or organisms derived from SB. "Omics" technologies refer to high-throughput technologies enabling the parallel analysis or profiling of various kinds of macromolecules such as DNA molecules in genomics, transcripts in transcriptomics, proteins in proteomics and metabolites in metabolomics. Technical aspects in collecting "omics" data sets are continuously improving and profiling techniques now serve several distinct purposes. "Omics" can provide complementary tools to study potential intended or unintended differences between GMOs and their comparators (e.g. in nutrient, anti-nutrient, endogenous toxicant or allergen levels) or to characterize the GMO's responses to environmental factors. However, the current value of "omics" data in risk assessment is limited since a considerable part stays uncharacterized and genomes, transcriptomes, proteomes and metabolomes are far from being thoroughly understood. Collecting data will be valuable provided that tools are at hand to interpret and understand them in a proper way.

Contrary to the expectations that the developments in pathway engineering will increase the complexity of biosafety permit applications due to the number of interactions to be assessed, it was argued that the "quantity of changes" regarding metabolic pathway engineering should not be overestimated in terms of introducing additional hazards.

The natural chemical space is so large and still in parts undiscovered that it is probable that so-called "new" metabolites are already produced in nature. Another point of view was that while the scale and speed at which new and complex organisms will be generated might considerably increase, our knowledge of new systems may not increase just as fast. From the risk evaluators' point of view, this could challenge the case-by-case approach in the future since they might be confronted with an increasing number of dossiers, each with an increased complexity. This could necessitate replacing the case-by-case approach with a more generalized assessment of groups of different products or organisms developed in SB. Within the context of contained use applications, a possible way forward to partly reduce the burden for risk evaluators would be to distinguish between applications that necessitate a comprehensive risk assessment and those applications that can generally be regarded as safe. More particularly, the question was whether a regulatory mechanism could be applied, which allows specific microorganisms to be exempted from some parts of Directive 2009/41/EC, provided they can be shown to be safe and to fulfil a given list of criteria (cfr Part 1 (b) of Article 3 within the Directive). Alleviating unnecessary regulatory burden of selected technology applications could foster innovations.

Currently developed protocells or protocell-like systems should be considered as chemical matters rather than living organisms. Accordingly, as for most systems currently assessed for potential chemical risks, data requirement should essentially be focused on the way they are assembled. Most participants agreed that these systems are currently not covered by the GMO regulation due to their inability to replicate. Future developments in the field will have to consider whether these systems are sterile or latent, and will demand an assessment of their capacity of replication and transfer of their own genetic material. Since current protocell developments will take place in chemical rather than in biological laboratories, there were also concerns as to whether developments can be properly monitored and regulated. Some participants made the parallel with nanotechnology, which is regulated at the level of applications rather than on the basis of the technique. Taking into account that protocells are currently essentially a model for basic research, the question of whether these systems are capable of evolution was judged premature. It was also noted that this field should not be overregulated due to their inability to propagate and the limited (if any) risks for human health and the environment.

Notwithstanding the fact that protocells and protocell-like systems are not likely to confer specific hazards in the short term, some participants opined the necessity for GMO safety advisory committees to evaluate them on a case-by-case basis. In their view, criteria for assessment could be based on the potential to confer risk rather than focusing on the properties of living organisms. For example, referring to prion-like proteins that transmit and propagate misfolded states of proteins (tau aggregates as an example), it was noticed that aspects of transmissibility and propagation of "information" could necessitate an assessment of protocell-like systems even when no genetic material is present.

With regard to *xenobiology*, most of the exchanges during the discussion concurred with the observation that applications in this field are still far away. Two main perspectives were brought up: some participants opined that xenobiology will use small modifications to develop products with new beneficial properties, whereas others claim that xenobiology can also have a much larger impact in the future as new artificial biosystems are created, thus adding a new level of complexity to nature. For the future, a proper assessment of potential interactions between organisms generated by means of xenobiology and natural organisms was identified to be crucial, even though xenobiology may give rise to organisms that are not regulated under the current GMO regulation. The need to characterize SB organisms in terms of their interaction (e.g. competitiveness) with natural organisms was a recurrent issue for most of the approaches discussed.

An application that would need consideration in the short term is the use of *minimal genomes* that serve as 'chassis genomes' to be expanded by genes not present in the parental genome. Such chassis organisms created for industrial purposes are usually generated from non-pathogenic organisms or organisms with a negligibly low pathogenicity. Moreover, it was noted that most of these organisms are expected to be auxotrophs and thus unlikely to propagate outside defined laboratory conditions. Another field of research consists in genome minimization aiming at exploring the smallest number of genes necessary for a cell to survive. Most of the participants opined that this approach is unlikely to generate organisms that are more pathogenic than their respective parental organisms but the potential deletion of genes involved in pathogenicity or virulence will remain a specific point of attention in the risk analysis. Within this regard, some examples were brought up where the pathogenicity of the resulting organisms was increased upon the deletion of single genes, thereby

illustrating that the deletion of single genes could sometimes lead to otherwise silent but deleterious properties. It should be noted that the increased efficiency of an organism's functions in consequence of genomic deletions is not specific to SB or genetic modification, nor does it by definition encompass an increased risk. One of the participants also emphasized that minimal organisms should not only be assessed in terms of their potential pathogenicity but should also be evaluated for other potentially changed properties (e.g. their possible environmental impact, as done for GMO risk assessment).

4.2 Is the GMO regulatory framework applicable to all fields of SB?

As a significant part of SB research is based on genetic modification techniques, it was generally assumed that SB should be regulated under the GMO regulatory framework. One participant suggested that the ongoing discussion on SB regulation could help to establish a "better" regulatory frame for GMOs, as the decision on how to regulate a new technology had significant consequences on the development of this technology. One possible way to reach this goal could be to exempt certain SB organisms from the Directive 2009/41/EC as already mentioned above. Another view was that products of SB should be regulated based on the resulting product and not on the process by which they have been developed.

Yet, it was remarked that creating a special status for SB would mean to overestimate that field which is at the moment firmly rooted in GM technology, whereas others emphasized that attention should be paid not to underestimate SB and its fast-paced scientific developments which could exceed GM technology, making necessary amendments to the existing regulatory framework.

In the past, the SB community proposed a system of self-regulation, meaning that scientists themselves should develop and adapt appropriate guidelines for risk assessment and risk management of their research. This approach was doubted to succeed, as concerns were raised that systems of self-regulation can only work until they are too time- or money-consuming and stand in the way of commercial interest.

During the discussion, no concrete examples were identified where current research may not be covered by GMO legislation, except for protocells, whose present developments are likely to fall within a regulatory framework covering chemicals rather than within the current GMO regulatory framework.

Yet, challenges to the regulatory framework may well lie ahead in the future. Xenobiology, with the modification of basic chemistry underlying genetic information, is likely to generate a specific challenge and a regulatory status on its own, unless the current GMO regulatory framework is amended to include this new type of modification.

5 Conclusions and perspectives

From the early 2000s on, when many scientists assigned their field of research with the contemporary significance of 'SB', the definition of the field has been subject to debate. Today there is still no internationally agreed consensus about the definition. The speakers of the workshop "Risk assessment challenges of Synthetic Biology" identified the developments in SB as a tool to introduce innovative elements and broaden the perspectives of potential applications in their specific domain of research. The lack of an internationally agreed consensus definition of SB should form in no case an obstacle to discuss potential risk assessment challenges. We are of the opinion that we should be careful assigning 'new' hazards to approaches of SB although this multidisciplinary field may give rise to an additional level of emerging and unintended hazards in the future that need further exploration.

Current developments in SB mainly involve the use of well-characterized microorganisms and genetic material and focus on research and development or on commercial production of substances in contained facilities. Sufficient knowledge and appropriate comparators are available and the current GMO risk assessment methodology provides a good framework to assess potential risks. Based on the experiences of our national advisory bodies and the results of the workshop, it is hardly conceivable that microorganisms or entities will be generated in the next few years that are far different from existing organisms. Therefore, the manipulation of synthetic organisms in the laboratory or their accidental release in the environment are unlikely to represent additional risks in the near future. This conclusion is in line with earlier reports (Pauwels et al. 2012; COGEM 2013; DFG, acatech and Leopoldina 2009; ZKBS 2012).

In the long term, developments in SB could generate organisms that will differ more fundamentally from naturally occurring ones. Several potential challenges to procedural elements, general principles and/or criteria in the current GMO risk

assessment methodology can be distinguished, based on the outcome of the discussion during the workshop.

One such general principle potentially challenged by developments in single subfields is the *comparative approach* of risk assessment. It could be more difficult to identify an appropriate 'natural' comparator in cases where unknown artificial sequences or complex combinations are used (pathway engineering), xeno-molecules or orthogonal systems are employed (xenobiology) or different cellular functions and cellularities are reconstituted (protocells). New organisms could be generated that are fundamentally different from those found in nature. The more an organism departs from a host or donor organism, the more difficult it will prove to assess the characteristics of the organism based on the characteristics of the different single parts of the host/donor organism. For example, it could become increasingly difficult for applications in the field of metabolic pathway engineering to assess the interactions between all novel parts/circuits. In those cases, the novelty and complexity of the resulting organism will demand a more comprehensive assessment. This might include the gathering of relevant information related e.g. to its potential pathogenicity, possible toxic or allergenic effects or its capacities for survival, multiplication and dispersal in potential receiving environments. Furthermore, the increase of scale and speed in SB applications, for example with high-throughput technologies, may impede the *case-by-case approach* of risk assessment from a practical perspective. This might present a possible pitfall for the regulatory framework, as it might challenge risk assessors, both from the point of view of having enough workforces to deal on a case-by-case basis with a greater number of applications and of the rising complexity of the genomic changes.

On the other hand, we do not expect the comparative approach to be challenged in other subfields of SB such as genome minimization, insertion of a (limited number) of well-characterized genetic circuits using isolated and characterized 'standard biological parts' or reconstitution of known microorganisms.

Regarding data required for performing a thorough risk assessment, and similar to the assessment of GMOs, the relevance of establishing "omics" profiles to SB developments could be considered. We think that the development of standardized and validated methods is a prerequisite and that the interpretation of data necessitates a good insight in the baseline of natural variations. Ideally, in order to beneficially use profiling techniques in risk assessment, it will be crucial to identify the appropriate questions and to tackle the potential gaps of data, in other words to distinguish what is "nice to know" from what is "needed to know".

5.1 Dealing with uncertainties

During the workshop, uncertainty was repeatedly put forward as a potential issue for future applications of SB. It is possible that the interaction between novel parts and circuits within organisms or the interaction of novel organisms with their environment will not be completely understood. Uncertainty is inherent to the concept of risk, hence risk assessment often deals with uncertainties that may arise from limitations or lack of data like limited exposure data, inadequacy of study design or model systems or different interpretations of existing data. This uncertainty can be addressed by gathering more information or by implementing appropriate risk management strategies. Existing risk management strategies, such as the division into risk groups, biological and physical containment and a precautionary attitude towards introduction into the environment, are applicable to most applications of SB. While one strategy to deal with uncertainty could be to adopt high levels of containment for organisms for which the risk assessment proves to be complex and associated with high levels of uncertainties, we are of the opinion that this should be done in a realistic and proportionate manner in order not to hamper research and only if there is sufficient reason to assume that the organism might have a higher risk potential.

Addressing uncertainty concerns is even more challenging when SB applications are proposed to be released in the environment. As for any other GMOs, organisms developed in SB should first be characterized in contained use to gather relevant scientific information while minimizing potential risks for human health and the environment. This containment can be gradually decreased if the evaluation of data shows that potential risks for human health and the environment are acceptable. Relevant data for the environmental risk assessment of these applications should include information on the physiology of synthetic organisms, their survival, their competing and/or evolutionary potential in receiving environments and their ability to exchange genetic material with other organisms. The collection of these environmental data will be crucial but challenging if organisms are very different from natural

ones and have only been studied in contained facilities. It is also possible that applicants will propose novel biosafety tools to mitigate potential risks. For example, orthogonality/xenobiology is presented by some researchers as the key to biosafety issues because it aims at preventing any exchange of genetic information with the natural world ("genetic firewall"). Since there are many uncertainties as regards the capability of the corresponding organisms to adapt, interact and evolve, we are of the opinion that these applications should be carefully looked at before xenobiology could be regarded as a technique enabling biological self-containment.

Finally, as in GMO risk assessment, remaining risk-related uncertainties following the risk assessment of SB applications should be addressed through appropriate monitoring through well-defined surveillance plans.

Regarding *regulatory frameworks*, we conclude that current activities involving the development and use of synthetic organisms make use of techniques that fall within the scope of Directives 2009/41/EC and 2001/18/EC. Some legislations of contained use of GMOs also cover non-GMO pathogens (e.g. Belgian regional decrees). In that case, the reconstitution of pathogenic microorganisms not differing genetically from their pathogenic archetype also falls within the provisions of the GMO regulatory framework. However, it should be noted that the use of protocellular systems unable to replicate or the modification of the basic chemistry underlying the genetic information machinery and processes could raise potential issues as regards the regulatory status of the resulting products or organisms. Along with the further development of these approaches, questions will be raised as to whether the understanding and/or definition of "organism", "GMO" or "genetic material" needs to be expanded or reconsidered to include these activities under the scope of the GMO regulatory framework. Alternatively, while protocells may fall under regulatory frameworks covering chemicals, the chemically modified products of xenobiology may fall under a new, specific regulatory framework.

5.2 Perspectives

This workshop, bringing together participants from fifteen European countries, representatives from EFSA and the European Commission, has given an appropriate setting to allow fruitful exchanges between scientists involved in research and development, experts involved in risk assessment/evaluation, and regulators involved in risk management. We are convinced that communication between scientists and risk assessors is crucial to timely identify emerging challenges for risk assessment and to estimate which information is relevant for the risk assessment. This is of particular relevance given the multidisciplinary and international character of SB, and the possibility that advances in high-throughput technologies of genetic modifications will soon enable developments in this field that may outpace the increase of knowledge on the risk potential of the organisms created.

As SB research becomes more complex or more distant from what we know as 'natural', mutual learning processes between these groups will be crucial to avoid overregulation (based on risk perception), which might result from a lack of understanding or from assigning new technologies with new hazards. Overregulation could lead to the application of unnecessary precaution and could signify a burden to the development of applications that may be beneficial for society. Therefore, while recognizing that a precautionary approach is important in cases of high complexity and uncertainty, we are of the opinion that application of containment, confinement, mitigation measures and monitoring should be realistic, proportionate to risk and adopted on a case-by-case basis to allow sufficient flexibility for research and development initiatives.

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