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# Scientific Opinion on Risk Assessment of Synthetic Biology

Michelle M. Epstein[1] and Theo Vermeire[2,‡,*]

**In 2013, three Scientific Committees of the European Commission (EC) drafted Scientific Opinions on synthetic biology that provide an operational definition and address risk assessment methodology, safety aspects, environmental risks, knowledge gaps, and research priorities. These Opinions contribute to the international discussions on the risk governance for synthetic biology developments.**

## The Context of the Scientific Opinions

In October 2013, three EC Directorates requested DG Santé Scientific Committees to provide Scientific Opinions on synthetic biology. The EC acknowledged that synthetic biology comes with promises of substantial benefits for health, the environment, resource management, and the economy, when fully based on a precautionary approach. It should avoid any harmful impact on the environment and emphasise conservation, sustainable use of biological diversity, and human health. The request for these opinions was motivated by the uncertainties and potential risks associated with synthetic biology (http://ec.europa.eu/health/scientific_committees/docs/synthetic_biology_mandate_en.pdf). The terms of reference pertained exclusively to safety and excluded human embryonic research or social, governance, ethical, and security implications. Opinion I [1] proposed an operational definition for synthetic biology (Box 1); provided an overview of the main scientific developments, concepts, tools, and research areas in synthetic biology (Box 2), the relation between synthetic biology and genetic engineering, and the possibility of distinguishing them; and summarised relevant regulatory aspects. Opinion II [2] addressed risk assessment methodology and safety aspects, and Opinion III [3] focused on risks to the environment, identified major gaps in knowledge for performing a reliable risk assessment, and provided research recommendations (Box 3). Here, we summarise these Opinions and describe them in their scientific and regulatory context.

## Synthetic Biology Compared with Genetic Modification

An operational definition of synthetic biology is presented in Box 1. Although synthetic biology uses genetic modification (GM) technologies, criteria such as the complexity of the GM, the speed of the modification, the number of independent modifications, or the degree of computational design are unable to unambiguously differentiate synthetic biology from GM. Synthetic biology is currently encompassed within European Directives 2001/18/EC [4] and 2009/41/EC [5] for genetic modification, thus making it possible to take advantage of current risk assessment methodologies and safety guidelines for GM work.

## Implications for Human Health and the Environment

Opinion II addressed the implications of likely developments in synthetic biology on human and animal health and the

### Box 1. The Operational Definition

'Synthetic biology is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms' [1].

This definition was derived from a working understanding of synthetic biology as a collection of conceptual and technological advances, enables risk assessment, and is sufficiently broad to include new developments in the field. By contrast, a survey of published definitions indicated that current definitions generally emphasise modularisation and engineering concepts as the main drivers for faster and easier GMO design, manufacture, and exploitation. To address this issue and enable practical work on risk assessment, the synthetic biology definition is operational, allowing the panel to unequivocally decide whether an activity is synthetic biology based or science based, which may change as synthetic biology concepts, tools, and applications evolve. 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. In comparison, synthetic biology includes any activity that aims to modify the genetic material of living organisms as defined in the Cartagena Protocol on Biosafety and does not exclude nonviable, nonreproducing goods, and materials generated by or through the use of such living genetically modified organisms (GMOs).

### Box 2. Six Novel Synthetic Biology Developments

The Opinions focus on the following six relatively new research areas of synthetic biology [2] and, in addition, the growing area of citizen science, all considered the most relevant for answering questions on the risks associated with synthetic biology:

- Genetic part libraries and methods: 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.
- Minimal cells and designer chassis: an alternative approach to the construction of industrial microbes.
- Protocells and artificial cells: 'bottom-up' approach that attempts to construct new simple forms of living systems using chemical and physical processes. A protocell is a basic cell type that comprises a RNA replicase and a fatty acid membrane; protocells have the possibility of becoming autonomous and integrating into existing organisms.
- Xenobiology: exploits nucleic acid analogues (e.g., xeno nucleic acids) as orthogonal information carriers unusable by natural biological systems and changes the genetic code by reprogramming the codon–amino acid table to expand the repertoire beyond the canonical 20 amino acids.
- DNA synthesis and genome editing: chemical synthesis of DNA to assemble constructs for introduction into living organisms that can enable genetic modifications in higher animals within a single generation.
- Citizen science: due to cheaper equipment, simpler, and easier methods and technologies, do-it-yourself biologists are increasing in number in a field traditionally reserved for highly trained professionals.

Box 3. Research Recommendations

The scientific committees were asked to review the state of the scientific knowledge concerning specific risks to the environment and biodiversity, address the major gaps in knowledge necessary for performing a reliable risk assessment, and provide research recommendations to ensure data quality and comparability, and the usability of the results for risk assessment. The recommendations in Opinion III are listed for each of the six novel synthetic biology developments and are intended to advise the EC on priorities for research funding. Here, we summarise the research recommendations that will have the greatest impact on the risk assessment methodology for synthetic biology:

- Developing computational tools to predict emergent properties of synthetic biology organisms (e.g., characterise the function of biological parts and predict their potential failure modes and interactions).
- Streamlining and standardising the method for submitting genetic modification data (e.g., submitting genetic parts information across EU member states to risk assessors that is transparent and available to all stakeholders).
- Developing reliable predictive tools for emergent safety issues at the systems level, preferably at the design stage.
- Encouraging the use of GMOs with proven safety records as comparators for risk assessment to ensure that the baseline state of safe organisms can advance step-by-step 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. By contrast, use of GMOs with a record of safety may better reflect the current understanding of risks.
- Developing sophisticated risk assessment tools to match the advances in technology and developing guidelines for risk assessors on evaluating potential emergent properties of genetically engineered systems. This should avoid an imbalance between risk assessment and technology that might negatively impact economic and health benefits of the technology and jeopardise the quality of safety protections.
- Improving the mechanistic understanding of underlying principles of containment and developing standardised techniques to monitor biocontainment and survival in environments outside the bioreactor and to generate comparative data for use in quantitative biocontainment assessment.
- Developing strategies to increase awareness and compliance of citizen scientists with national biosafety rules and codes of ethics, including collaboration with acknowledged institutions and training.

environment. Directives 2001/18/EC and 2009/41/EC and the Guidance notes published in Commission Decision 2000/608/EC [6] addressed the magnitude and probability of potential hazards and adverse effects of genetic engineering to human health and the environment. New challenges in predicting risks are expected due to emergent properties of synthetic biology, including the integration of protocells, future developments of autonomous protocells, use of nonstandard biochemical systems in living cells, increased speed of modifications by new technologies for DNA synthesis and genome editing, and a rapidly evolving do-it-yourself biology (DIYbio) and citizen science community.

## Risk Assessment Methodology for Synthetic Biology

Opinion II also addressed whether existing European Union (EU) risk assessment practices for genetically modified organisms (GMOs) are adequate for synthetic biology and suggested revising risk assessment methods and risk mitigation procedures regularly because of the rapid evolution of synthetic biology technology. New risk assessment approaches may be necessary to tackle risks pertaining to routes of exposure and adverse effects arising from new technologies leading to unfamiliar biological entities, such as the integration of protocells into living organisms, xenobiology, and DNA synthesis and direct genome editing of zygotes (Box 2). Such modifications may be multiplexed GMs, with increased modifications introduced in parallel by large-scale DNA synthesis and/or highly parallel genome editing, with increased genetic distance between the resulting organism and any natural or previously modified organism. Therefore, it is important to ensure continued safety protection proportionate to the risk of new, unknown, and unexpected organisms that arise from synthetic biology, and risk assessment methods must advance in parallel with synthetic biology advances.

To address this risk, safety locks, which are biological containment strategies meant to prevent the dissemination and contamination of synthetic biology organisms, are essential. Some examples include induced lethality ('kill switches'), horizontal gene transfer prevention, trophic containment (auxotrophic organisms designed incapable of synthesising a compound required for survival that cannot be found outside a controlled environment), and semantic containment [7]. However, such strategies are not yet sufficiently reliable or robust for the field release of engineered bacteria because of mutation and positive selection pressure for mutants that may escape safeguards. A general strategy for designing inherently safe applications for synthetic biology is demanding because of the stochastic and probabilistic character of the underlying biochemical synthetic biology processes. Importantly, there is no single reliable safety lock technology at present.

## Specific Risks to the Environment

The impacts on biological diversity and conservation were analysed in Opinion III. Specific risks to the environment may occur secondary to accidental release, persistence of synthetic biology organisms designed for environmental release, synthetic biology organisms becoming invasive or disrupting food webs, transfer of genetic material from vertical gene flow or horizontal gene transfer, and potential impacts on biodiversity and ecosystems from 'de-extinction'. Importantly, the uncontrolled ecological release of synthetic biology organisms with potentially negative impacts on biodiversity and conservation was not considered a risk for the next 10 years (to 2025), because the technology that would allow viable synthetic organisms to escape to the environment is not yet sufficiently advanced. Risk prevention for synthetic biology organisms considers many potential strategies, including designing less-competitive organisms by changing metabolic pathways, replacing metabolic pathways with built-in dependencies on external biochemicals, designing evolutionarily robust biological circuits, using biological systems based on an

alternative biochemical structure to avoid gene flow to and from wild species, and designing protocells that lack key features of living entities, such as growth and replication. Organisms not retrieved once released or escaped into the environment require risk mitigation: risk reduction measures after deliberate or accidental release of synthetic biology organisms, components, or products, and after all biocontainment processes, safety locks, and other preventive measures have failed. In high-risk cases, risk mitigation may require a coordinated, efficient, and proportional international response and implementation of WHO International Health Regulation standards, including the prior assessment of the necessity for international notification.

## Knowledge Gaps

Opinion III also focused on knowledge gaps that impede reliable risk assessment for synthetic biology risks on human health and the environment for the six novel synthetic biology developments (Box 2). For genetic parts, tools for predicting emergent properties of complex biological systems may not be accurate or available to risk assessors; methods for submitting GM data and genetic parts information to risk assessors are unstandardised and might limit risk assessment. For minimal cells and designer chassis, it is important to define and engineer biological robustness to move closer to neutral or even zero evolution. For protocells, there is little information about the behaviour, impact, and evolutionary ramifications of interactions of systems comprising organisms and chemical nonliving systems. There are unknown hazardous properties of future autonomous, replicating chemical systems, including allergenicity, pathogenicity, and biological stability. Additionally, there is a lack of knowledge on the behaviour of 'natural protocells', such as lipid vesicles produced by bacteria and loaded with peptides, RNA, or DNA, which may be comparable to synthetic protocells. For xenobiology, there are unknown adverse effects of nonstandard biochemical molecules and/or systems in living cells and of novel xenobiological compounds. There is a lack of data supporting risk assessment regarding the change in evolutionary fitness; ecological competitiveness; degree of horizontal gene flow; or susceptibility to viruses, diseases, or predation. For DNA synthesis and genome editing, increased speed of modification might challenge risk assessment because administrative procedures might not be able to cope with a large number of rapidly created engineered organisms. Critically, for all areas of synthetic biology, a sufficient mechanistic understanding of the underlying principles of containment is necessary, such as using different genetic codes or alternative biochemistries of biopolymers, such as nucleic acids or amino acids. A reliable metric to measure the escape frequency of synthetic biology organisms is also required. A clear strategy is recommended for new forms of biocontainment and additional layers of containment using orthogonal systems.

## Beyond the Opinions

The Opinions provide a synthetic biology operational definition. We acknowledge that the Opinions conclude that the existing risk assessment methodologies for GMOs currently are applicable to synthetic biology. However, as the synthetic biology field evolves, the definition and risk methodologies should be re-evaluated. These Opinions are in the public domain and are used by the EC and other international institutions to support regulatory and policy decisions; for example, the Opinions were the basis for the proposed definition of the Ad Hoc Technical Expert Committee of the Convention on Biological Diversity [8] and will support the preparation of the EC position for the Convention on Biological Diversity in December 2016 (COP13). The Opinions will also contribute to the development of a risk governance approach for synthetic biology by the EC and potentially at the international level focusing on public and political acceptance, such as social, ethical, and security implications. Importantly, we emphasise that advances in synthetic biology must be closely monitored to avoid potential problems affecting health, the environment, and biodiversity. Moreover, it is necessary to consider alternative risk assessment methods to cope with a lack of comparator organisms and focus on containment strategies to prevent the uncontrolled escape and deliberate release of synthetic biology organisms.

[1]Medical University of Vienna, Vienna, Austria
[2]National Institute for Public Health and the Environment
[†]The authors are rapporteur and chair, respectively, of the Working Group on Synthetic Biology of the three non-food Scientific Committees of the European Commission: SCENIHR, SCHER, and SCCS http://ec.europa.eu/health/scientific_committees/index_en.htm. The other members of this Working Group are: P. Hartemann, A. Prokovya, L. Martinez Martinez, E. Rodriguez Farré (all SCENIHR); T. Fernandes (SCHER); Q. Chaudhry, M. Dusinska, T. Platzek, S.C. Rastogi, J. van Benthem (all SCCS); and the external experts R. Breitling, J. Bridges, C. Delebecque, T. Gardner, K. Pauwels, J. Philp, M. Schmidt, and E. Takano.

*Correspondence: theo.vermeire@rivm.nl (T. Vermeire).

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