HSE

Health and Safety Executive

Synthetic biology

A review of the technology, and current and future needs from the regulatory framework in Great Britain

Prepared by the **Health and Safety Laboratory**
for the Health and Safety Executive

RR944
Research Report

HSE

Health and Safety Executive

Synthetic biology

A review of the technology, and current and future needs from the regulatory framework in Great Britain

Claire Bailey
Heather Metcalf
Brian Crook
Harpur Hill
Buxton
Derbyshire
SK17 9JN

Synthetic biology has been described as 'the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes'. It encompasses engineering of DNA-based biological circuits using standard biological parts, finding the minimal genome capable of functioning, constructing protocells, ie, living cells from scratch, and chemical synthetic biology in which biological systems are created based on a biochemistry not invented by evolution (ie, not the G-C-A-T nucleic acid backbone). It has developed from genetic technology that has discovered the functions of genes and proteins to enable the above developments, assisted by supporting technology that now allows gene sequencing to be done more quickly and at a much lower cost, as well as the emergence of commercial sequencing services and standardised production of genetic sequences, or 'Biobricks'. The consequence of this development of the technology is to make it more widely accessible and expand it beyond microbiology into the disciplines of engineering, chemistry and computing.

This report and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

HSE Books

First published 2012

KEY MESSAGES

Synthetic biology has been described as “the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes”. Potential applications include more efficient or inexpensive drug production and developing new renewable energy sources, micro-organisms for environmental remediation of pollutants, and biosensors to detect infections in hospital patients.

This project by HSL aimed to review for HSE the current status and potential applications of synthetic biology and approaches to biological risk assessments. The technology is at an early but rapidly developing stage, therefore it is timely to determine whether it presents any health and safety risks that are not covered by existing legislation. Recent reports have summarised the legislative position regarding synthetic biology in the EU in general, in Holland, and in USA, while other reports have provided a detailed examination of synthetic biology bioethics and biosafety. Synthetic biology is at present covered by Genetically Modified Organisms regulations, which provide a framework for risk assessment and notification for laboratories undertaking GM work and therefore also synthetic biology. Future possible changes to biological agents regulations in Britain would leave the GM regulatory framework largely unchanged. Although fundamentally similar to genetic modification, in which the known traits of each parent organism can be used to assess the characteristics of the created novel organism, with synthetic biology in theory an organism can be constructed from individual genetic sequences. Therefore the characteristics of the parent organism are less obvious, making assessment of traits difficult. Because synthetic biology uses a wider range of disciplines such as engineering and computation scientists, it may mean that those working with synthetic organisms are less familiar with microbiological risk assessment. However, other disciplines may bring new approaches to risk assessment. Ultimately synthetic biology may be applied outside the laboratory, for example in contaminated land remediation. Consequently, a robust knowledge of the characteristics of a synthetic organism would need to be known before it could be considered for controlled release.

Because synthetic biology is a developing technology, few examples of its practical application currently exist, although there are a number of proof-of-concept studies. The U.S. based International Genetically Engineered Machine Competition (iGEM) is held every year, in which university teams work to develop novel applications for synthetic biology. This competition has grown rapidly to 130 participants in 2010, and teams from British universities regularly participate with notable success.

In theory, synthetic biology can offer many benefits to society but raises ethical issues. However, evidence from public debates on synthetic biology suggests it is viewed more positively than other genetic modification, possibly because information on current research is more openly available to the public, that positive benefits are being clearly presented, and that they are seen to be of general benefit rather than providing profit for commercial companies.

In summary, the current regulatory framework for GMOs in Britain adequately covers present and near future synthetic biology activities, but it will be important to maintain a watching brief on new developments so that HSE, as the regulatory body, will be equal to the challenge of reviewing project notifications and risk assessments. This review provided details of current approaches to biosafety risk assessment and the potential future challenges that might need additional guidance or more detailed independent assessment from the regulator. Using the precautionary principle would dictate that work with synthetic agents is only conducted in high containment facilities. This would be disproportionate, but researchers must provide robust and clearly argued risk assessments. It will be important for regulators and dutyholders to develop and maintain a dialogue to ensure that this procedure is put in place.

EXECUTIVE SUMMARY

Synthetic biology has been described as "the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes". It encompasses engineering of DNA-based biological circuits using standard biological parts, finding the minimal genome capable of functioning, constructing protocells, i.e., living cells from scratch, and chemical synthetic biology in which biological systems are created based on a biochemistry not invented by evolution (i.e., not the G-C-A-T nucleic acid backbone). It has developed from genetic technology that has discovered the functions of genes and proteins to enable the above developments, assisted by supporting technology that now allows gene sequencing to be done more quickly and at a much lower cost, as well as the emergence of commercial sequencing services and standardised production of genetic sequences, or 'Biobricks'. The consequence of this development of the technology is to make it more widely accessible and expand it beyond microbiology into the disciplines of engineering, chemistry and computing.

Potential applications of the results of synthetic biology research include more efficient or inexpensive production of drugs; development of new renewable energy sources using modified micro-organisms; developing micro-organisms that can speed up environmental remediation of toxic pollutants; and development of biosensors to detect infections in hospital patients. Although many research groups are working on synthetic biology globally, including several mainly university based groups in Britain, the technology is still in its infancy. Therefore it is timely to review the technology and to determine whether it presents any health and safety risks that are not already covered by existing legislation. In this respect, the potential complications are that, although fundamentally similar to genetic modification, the difference may be summarised as 'top down' versus 'bottom up'. Typically with genetically modified organisms, genetic sequences from a donor organism are inserted into a recipient organism. Therefore the traits of each parent organism are known and can be used to assess the characteristics of the created novel organism. With synthetic biology, in theory an organism can be constructed from individual genetic sequences. Therefore the characteristics of the parent organism are less obvious, making assessment of traits difficult. Because of the broader use of the technology by disciplines such as engineering and computation scientists, it may mean that those working with synthetic organisms have a less deep understanding of microbiology and be less familiar with microbiological risks, or the risk assessment process. However, the converse of this is that other disciplines may bring new approaches to risk assessment. The ultimate aim of synthetic biology is the application outside the confines of the laboratory, for example in contaminated land remediation. Consequently, a robust knowledge of the characteristics of a synthetic organism would need to be known before it could be considered for controlled release.

This project by HSL aimed to review for HSE the current state of the art and the potential applications of synthetic biology, including its application beyond the traditional biological agents sector. This includes what approaches are currently being used to develop biological risk assessments. The information obtained can then be used to assist in devising a proportionate regulatory approach to a new and emerging technology, as well as the need for possible future guidance or regulation, and the resources likely to be required in future for HSE's Biological Agents Unit to regulate the sector. A further consideration is the potential use of synthetic biology applications by users outside the conventional laboratory discipline, often termed 'DIYBio' or 'Garage Biology'. While outside HSE's remit to regulate, there is a need to know if such activity could impact on public perception of the technology that might influence industrial application.

Key words were identified with which to search online literature databases, supplemented by searches by HSL Information Centre knowledge management staff to access the broadest

possible range of peer reviewed papers and other reports. The review focussed on aspects of risk assessment but also included ethics and public perception.

Because synthetic biology is a developing technology, few examples of its practical application currently exist, but most notable is a U.S. project to engineer a yeast to produce artemisinic acid, the precursor for artemisinin, an anti-malarial drug. Another example is work to engineer a bacterium to secrete spider silk proteins to create light and strong woven material. Beyond this there are a number of proof-of-concept studies based on developing technologies to make the engineering of biology more straightforward and reliable. These include work to produce an engineered genetic toggle switch in bacteria to become light sensitive and express fluorescent protein. The U.S. based International Genetically Engineered Machine Competition (iGEM) is held every year, in which university teams including biologists and engineers from around the world are encouraged to work together to produce new applications for synthetic biology. The participants receive standard biological parts ('Biobricks') which they use alongside parts of their own design to produce new biological systems that can operate inside living cells. This competition has grown rapidly from five teams in 2003 to 130 in 2010, and is generating a wide range of potential novel applications. Teams from British universities regularly participate with notable success.

In theory, synthetic biology can offer many benefits to society. However as a new technology that alters the fundamental code of life, ethical debate is ongoing focussing on whether it is acceptable to manufacture modified organisms that would not have evolved naturally. There is a concern that an escaping organism could threaten ecosystems. Other concerns include regeneration of previously existing pathogens, such as the Spanish Flu pandemic influenza strain, or the polio virus. While critics oppose the recreation of previously eradicated pathogens, conversely the associated research could lead to development of possible vaccines. With proper regulation and risk assessments, the consensus is that the likelihood of an untoward incident involving accidental or deliberate release of a modified organism could be significantly reduced. The management and regulation of modified organisms through risk assessment is therefore a priority. The attention to issues of safety and social consequences of synthetic biology has been described as "safety-by-design", an attempt to extend self-governance models developed in the early days of genetic modification. Examples include designing in genomes that maximise control over their function, thus minimising the risk of survival or re-programmability outside the laboratory.

Regarding unregulated 'DIYBio' or 'garage biology' synthetic biology activity, it has been suggested that there is an informal code of ethics for this 'biohacker' community that recognises not only the risk to their own health but the negative publicity of adverse outcomes.

Evidence from public debates on synthetic biology suggests that it is viewed in a more positive light than genetic modification, such as GM foods, despite the similarities. This may result from the current research being more open and information more available to the public, that positive benefits are being clearly presented, and that they are seen to be of general benefit rather than providing profit for commercial companies.

The robust risk assessments needed for synthetic biology work for occupational health protection and to address public health concerns are at present covered by Genetically Modified Organisms regulations. In Britain this is the Genetically Modified Organisms (Contained Use) Regulations 2000, which implement the European GM Micro-organisms (Contained Use) Directive (2009/41/EC). This provides a framework for risk assessment and notification for laboratories undertaking GM work and therefore also synthetic biology activities. Future possible changes to biological agents regulations would leave the GM regulatory framework largely unchanged. The main question is how this risk assessment process applies to synthetic

biology, not only for current work but also for future work for example with protocells, i.e., the building of organisms from bioparts where there are no parent organisms from which to assess biosafety characteristics.

A recent report from a European Commission committee has summarised the legislative position regarding synthetic biology in the EU, while a report from the Dutch GM legislative body COGEM reviewed in detail how their legal framework, risk assessment and risk management processes applied to synthetic biology. Other reports from OECD and the Royal Society, the International Association for Synthetic Biology, and a US Presidential Synthetic Biology Bioethics Commission have all included consideration of biosafety risk assessment, while other publications have specifically examined the risk assessment process for protocells. A number of publications by Markus Schmidt from the Organisation for International Dialogue and Conflict Management Biosafety Working Group, Vienna have provided a detailed examination of synthetic biology biosafety, and the author has also provided biosafety input to the iGEM competition.

The general view is that the current risk analysis system for GMOs also applies to synthetic organisms, although difficulties could arise in assessing the characteristics of an organism that has been created via the bottom-up approach. While the precautionary principle could be applied to dictate that work is conducted at high containment, this may present an unnecessary impediment to scientific and commercial progress.

Technological developments could result in greater understanding of genes and the possible interactions between gene products, so that risk assessment could become easier, with the risks associated with future activities becoming more predictable over time. A stepwise approach could be applied so that the first experiments are carried out on a small scale in the laboratory until sufficient data have been obtained. Once these data are available, the activities can be carried out on progressively larger scales with more data becoming available at each subsequent step. To overcome possible problems of new researchers with a professional background other than biology, who may be unfamiliar with handling biological material and the associated risk assessment, biosafety training dealing with risks and best practices will be important, also development of biosafety manuals specifically for synthetic biology laboratories, and broadening the review function of Institutional Biosafety Committees. Risk assessment tools from other scientific disciplines such as engineering (for example, Event – or Fault Tree Analysis) could add to the synthetic biology risk assessment process. Use of standardised, and therefore more predictable, bioparts (such as from the Biobrick registry) will be important to develop robust biosafety data.

In summary, the current regulatory framework for GMOs in Britain adequately covers present and near future synthetic biology activities, but it will be important to maintain a watching brief on new developments in technology so that HSE, as the regulatory body, will be equal to the challenge of reviewing project notifications and associated risk assessments. This review has provided details of current approaches to biosafety risk assessment and the potential future challenges that might require additional guidance or more detailed independent assessment from the regulator. Using the precautionary principle, to dictate that work with synthetic agents is only conducted in high containment facilities, could be considered disproportionate, but only if the researchers are able to provide robust and clearly argued risk assessments. It will be important for regulators and dutyholders to develop and maintain a dialogue to ensure that this procedure is put in place.

CONTENTS PAGE

1 SYNTHETIC BIOLOGY: INTRODUCTION AND BACKGROUND

1.1 OVERVIEW

Synthetic biology is a term used to cover areas of biochemistry research that is involved in the chemical synthesis of DNA, utilising biological agents or their components for potential application across a wide range of industrial sectors. The ethos of synthetic biology is to implement engineering, chemistry and computing principles of predictability and reproducibility into building biological systems. An authority on synthetic biology ethics, Schmidt (2009) has stated that while several definitions exist for synthetic biology, the one most frequently cited is "the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes." (http://syntheticbiology.org/).

Although this definition could equally apply to genetic modification, where it does differ is the implied greater use of engineering principles. This is explained in more detail by Schmidt, who subdivided synthetic biology as follows:

1. Engineering DNA based biological circuits, by using, e.g., standard biological parts;
2. Finding the minimal genome;
3. Constructing protocells, in other words, living cells from scratch; and
4. Chemical synthetic biology, creating orthogonal biological systems based on a biochemistry not invented by evolution.

These are described in more detail later in this report.

The following table, taken from Schmidt (2009), neatly summarises the main areas of synthetic biology.

Brief description of the four subfields in synthetic biology				
	DNA-based bio-circuits	**Minimal genome**	**Protocells**	**Chemical synthetic biology**
Aims	Designing genetic circuits, e.g., from standardised biological parts, devices and systems	Finding the smallest possible genome that can 'run' a cell, to be used as a chassis, reduced complexity	To construct viable approximations of cells; to understand biology and the origin of life	Using atypical biochemical systems for biological processes, creating a parallel worked
Method	Design and fabricate; applying engineering principles using Standard parts and abstraction hierarchies	Bioinformatics-based engineering	Theoretical modelling and experimental construction	Changing structurally conservative molecules such as DNA

Techniques	Design of genetic circuits on the blackboard, inserting the circuits in living cells	Deletion of genes and/or synthesis of entire genome and transplanting the genome in a cytoplasm	Chemical production of cellular containers, insertion of metabolic components	Searching for alternative chemical systems with similar biological functions
Examples	"AND" gate, "OR" gate; genetic oscillator; repressilator; Artemisinin; Metabolism, "Bactoblood"	DNA-Synthesis and transplantation of *Mycoplasma genitalium*	Containers such as micelles and vesicles are filled up with genetic and metabolic components	DNA with different set of base pairs, nucleotides with different sugar molecules

This area of research has developed from genetic technology that initially focused on deciphering the information encoded in genome sequences to discover the functions of genes and the proteins that they produced. As a direct consequence of the rapid developments in the methodology to read genes, genome technology can now be performed relatively cheaply. This has revolutionised the genetic industry and genes that at first took weeks to sequence now take days. Similarly, the cost of gene sequencing has also fallen. To some extent this has taken it away from specialised laboratory research done by a small number of individuals to a more standardised and commercial sequencing service and production of genetic sequences (sometimes referred to as 'Biobricks'). The consequence of this development of the technology is to make it more widely accessible and expand it beyond microbiology into the disciplines of engineering, chemistry and computing (Neumann and Neumann-Staubitz, 2010). We are already seeing research in a wide range of applications, a benefit to cross-field working. Current examples include:

- Making the production of drugs more efficient and inexpensive (Heinemann and Panke, 2006);
- Development of new renewable energy sources using modified micro-organisms (Balmer and Martin, 2008);
- Development of micro-organisms that can speed up environmental remediation, such as the breaking down of toxic pollutant chemicals in soil (Keasling, 2008; POST, 2008);
- Creation of disease and drought resistant plants which can withstand harsher environments (See activities of Synthetic Plant Products for Industry Network (SPPI-Net) and workshop report at http://www.sppi-net.org/downloads/MeetingSept09.pdf);
- Development of biosensors that detect developing infections in hospital patients (Neumann and Neumann-Staubitz, 2010).

Indeed, horizon scanning reviews of the technology (RAE, 2009) suggest that it could have a potential impact similar to that of the semi-conductor in the last century.

1.2 THE NEED FOR THIS REVIEW

In much the same way as the use of genetic modification previously raised concerns due to the perceived unpredictability of 'creating new life forms', synthetic biology is coming under similar scrutiny. As HSE is the regulator for work with biological agents, including contained use working with those that have been genetically modified, there is a need for the specialist microbiology inspectors in Biological Agents Unit to be aware of current and future developments in synthetic biology research and the potential risks that may need to be assessed.

The perceived potential complications are as follows:

- Although fundamentally similar to genetic modification, the difference may be summarised as 'top down' versus 'bottom up'. Typically with genetically modified organisms, genetic sequences from a donor organism are inserted into a recipient organism. Therefore the traits of each parent organism are known and can be used to assess the characteristics of the created novel organism. With synthetic biology, in theory an organism can be constructed from individual genetic sequences. Therefore the characteristics of the parent organism are less obvious, making assessment of traits difficult.

- The broader use of the technology by disciplines that may have a less deep understanding of microbiology may means that they are less familiar with microbiological risks, or the risk assessment process. However, the converse of this is that other disciplines may bring new approaches to risk assessment.

- The ultimate aim of synthetic biology is the application outside the confines of the laboratory, for example in contaminated land remediation. Consequently, a robust knowledge of the characteristics of a synthetic organism would need to be known before it could be considered for controlled release.

HSE needs to know the extent of the use of the technology, where it is being used in Britain and in what circumstances, and how centres using the technology approach risk assessment. The information obtained can then be used to assist in devising a proportionate regulatory approach to a new and emerging technology, as well as the need for possible future guidance or regulation, and the resources likely to be required in future for Biological Agents Unit to regulate the sector. At this time, only a relatively small number of centres use this technology, but it is likely to expand, therefore this is a timely opportunity to inform and influence the majority of users at minimal effort.

This literature review is the first stage of this project and aims to summarise the background to synthetic biology. It will look at the current state of the research within Britain and the potential applications, including those disciplines beyond the traditional biological agents sector. It also aims to highlight potential difficulties in assessing risk, and current risk assessment procedures. The technology is reviewed in the light of the current health and safety regulations regarding genetically modified organisms, and whether there is a need to modify the regulations in accordance with this emerging technology.

A second objective of the project is to compile a list of the establishments who are currently working on synthetic biology projects in Britain. This will be the subject of a separate report (HSL Report HEX/10/11).

A further consideration that will be touched on is the potential use of synthetic biology applications by users outside the conventional laboratory discipline, often termed 'DIYBio' or 'Garage Biology' (Ledford, 2010). While an individual undertaking work in a non-traditional laboratory setting will still be covered under the existing HSE remit (as the Regulations cover "a person" rather than the "employer", the difficulty is of knowing what activity is taking place. There is a need for HSE to know what the potential impact of such activity may indirectly have on occupational or public health, or indeed on the public perception of the technology that might influence industrial application.

A final objective of the project, following on from this report and the list of establishments planning or performing work in the synthetic biology area, will concentrate on the stratification of the health and safety risks of synthetic biology, using the data gathered from the previous stages of the project, to plan future need for guidance and HSE resource.

2 REVIEW METHODOLOGY

A state of the art review has been undertaken using appropriate search terms to obtain data from published reports and papers. The use of online search engines such as Medline, Web of Science and Google Scholar by the review team were supplemented by use of the HSL Information Centre to conduct a series of database searches which potentially provide broader access to 'grey literature', such as government reports not published through the traditional peer reviewed journal route.

Search terms used included the following phrases in combination and separately:

- Synthetic biology, genetic engineering, novel genetically modified organisms, DNA synthesis and synthetic genomics.

The following phrases were only used in conjunction with DNA synthesis, synthetic biology or synthetic genomics:

- Ethics, risk assessment, public perception, ongoing research and biological systems.

All papers from the searches were initially screened by title. The abstract was then read and sifted for those that appeared of relevance. Finally, the full paper was read if it was thought that it potentially contained sufficient useful information, also including those where it was not clear from the initial abstract but whose content seemed promising. The papers and articles were further classified into broad categories to aid analysis. All the papers found in the search are included in the bibliography and are ordered into relevant categories. Where particular reference to a paper is made in this report, the details are included in the reference list.

3 SYNTHETIC BIOLOGY PROCEDURES: THE PROCESS BEHIND THE TECHNOLOGY

Living systems are composed of a number of key components (cells, genes, proteins) that allow growth and replication. Understanding of how these components interact in living systems has formed the basis of biological and biomedical research over several decades.

All life forms are composed of molecules (proteins, lipids, sugars, DNA, RNA) that are, in themselves, non-living. However, the definition of life at a biochemical level is when these assembled molecules are able to continually regenerate, self-replicate and evolve. Regeneration and replication requires the living system to be able to import, process and transform molecules from the environment to create cells, while evolution requires heritable variation in cellular processes (RAE, 2009). Living systems have the mechanisms to achieve these requirements. The instructions for life are stored in informational chemical polymers (such as DNA and RNA) and these encode metabolic systems for chemical regulation and regeneration of components, all of which are achieved within a living cell; the simplest form of life. More complex forms of life comprise many cells working together in a coordinated and regulated manner with differentiation of function.

Underpinning this process is the ability of non-living molecules to self-organise (Lehn, 2002). With combinations of the basic chemical building blocks (Guanine, Cytosine, Adenine, Thymine in DNA or Uracil in RNA), strands of nucleic acids can pair up to form large DNA or RNA molecules and allow the storage and retrieval of information. Transcription is the process by which the genetic code (DNA) instructs cells to produce proteins via intermediary messenger RNA (mRNA), which is followed by translation. Translation occurs when the mRNA creates a polypeptide chain comprising a defined sequence of the 20 naturally occurring amino acids to produce proteins that carry out most of the cellular functions and activities within organisms. In effect, the three-letter encodement used at the genetic level in mRNA is 'bridged' by the adapter molecule transfer RNA (tRNA), in order to generate the amino acid sequence that ultimately leads to protein synthesis (Holley *et al*, 1965)

Key milestones in the development of current molecular biology research have been:

- Elucidation of the relationship between DNA, RNA and proteins by Watson, Crick and co-workers in the 1950s through discovery of the structure of the double helix.

- Elucidation by Brenner and colleagues in the 1960s of the role of messenger RNA (mRNA) in how DNA instructs cells to make specific proteins.

- Development of cloning techniques such as the transfer of genes into bacterial cells in order to reproduce and generate multiple copies.

- The development of radio-labelling (e.g., P^{32}) techniques for reading DNA chemical bases (DNA sequencing) leading to the initial sequencing of the human genome and subsequently the genomes of an increasing number of species.

Research has continually increased in sophistication from study at the population level to individual organisms, from the physiological level to the individual cell and, more recently, to the molecular scale. This later development has enabled study of individual biochemical reactions and metabolic pathways, gene regulation and the control of cell division and cell-cell signalling.

The traditional approach to biological research was to isolate a small number of biological components in order to understand their structure and function. This approach assumed that single biochemical events resulted in single effects, in a simple cause and effect relationship. However, most genes, proteins and other components operate by a complex network of regulated interactions, and simple cause and effect observations are insufficient. This led to the emergence of systems biology which combine biological measurements with mathematical and computational modelling to enable interactions between the components of a biological system to be explored to predict observed properties. This has only been possible because of technological developments, such as more powerful computers and supporting IT systems that has enabled genome sequences of hundreds of different organisms and their protein components to be identified and studied. The development of this largely automated technology, with rapid, high-throughput DNA sequencing developed in systems biology, has also led to the development of synthetic biology.

Synthetic biology may be considered as combining the principles of biology with physical sciences, engineering and computing. While systems biology aims to study natural biological systems as a whole and uses simulation and modelling tools in comparison with experimental information using measurement methods such as microscopy, flow cytometry etc., synthetic biology aims to build novel and artificial biological parts, devices and systems. Many of the same methods are used, but in synthetic biology the fundamentals of engineering is used, such as defining systems in terms of mathematical equations. Once a system, or part of a system, has been described in this way it can be reduced to its biological parts (bioparts) whose function is expressed in terms of input/output characteristics. Inventories or registries of these defined parts can be created and called upon for future use, much in the way an engineer would use standard components. A system designer can use these functionally characterised components and combine them into devices and, theoretically, into systems often with the aid of computer augmented design (CAD). At this level there is a fundamental difference from genetic engineering, where the systems biology approach may be used to define specific genes that are then modified to produce functional changes in the characteristics of the modified organism. In some approaches, this is done from scratch, rebuilding a biological system in a process called re-writing (Kaznessis, 2007).

The modular bioparts, as described above, are designed to be easily combined with other parts. The ultimate aim is to produce a range of standard devices (built from standard parts) which can be used in standard systems. Examples include DNA-based bio-circuits such as biologically based equivalents of the N/AND gates that are the basis of counters, calculators and computers in electronic devices.

Development of standard bioparts with consistent and defined function is being led by the BioBricks Foundation (*http://bbf.openwetware.org/*), a not-for-profit organization founded by engineers and scientists from the Massachusetts Institute of Technology (MIT), Harvard, and University of California. Information about the bioparts or BioBricks is stored on a Registry of Standard Biological Parts (*http://partsregistry.org/Main_Page*) run by MIT which is available to the public free of charge.

Other approaches in synthetic biology are to define the minimal genome, i.e., finding the smallest possible genome that can 'run' a cell and that can be used as a chassis to develop functioning organisms of reduced complexity.

This requires an understanding of, for example, protein-protein interactions and the functional importance of what may appear to be unrelated genes (Jones and Thornton, 2010). Work has been done on the bacteriophage T7 genome as a model for simple study (Chan *et al*, 2005). For a gene to be expressed there must be promoter sequences and the above mentioned proteins

present. If any are missing or modified, the organism may not function, i.e., express its genes to give a particular phenotypic function, as expected. This is particularly relevant when we consider the new organism designed and grown by Professor C. Venter (Gibson *et al*, 2010). The publicity surrounding the published research implied that a new organism had been developed and built from scratch. This would require the design of a completely new genetic code based on the existing four-base sequence. To maintain a safe organism, the researchers would have to make sure that this new genome did not contain any sequences that might code for virulence genes or factors. They would also have to engineer promoter sequences, and all the initiator enzymes. This is a considerable challenge even with current technology, and in reality, Venter's team used an existing organism with its own genome removed. This acted as a host for their DNA plasmid formed of genes with known sequences and functions, and in this respect was closely related to more conventional genetic modification.

While the development and combination together of bioparts may be considered as a 'bottom-up' approach to synthetic biology, other areas of synthetic biology research such as chemical synthetic biology or the development of protocells is working at an even more fundamental level. Researchers aim to use chemical approaches to build synthetic cells and biological systems from scratch. This would use chemical components that are not necessarily natural, but which mimic the properties of natural molecules and macromolecules. This presents a considerable challenge and is at an early stage of development, but the ultimate aim would be for these protocells to be self replicating (Szostak *et al*, 2001; Luisi *et al*, 2006).

4 THE APPLICATION OF SYNTHETIC BIOLOGY: ACHIEVEMENTS TO DATE INTERNATIONALLY AND IN BRITAIN

4.1 GLOBAL IMPACT OF SYNTHETIC BIOLOGY

The field of synthetic biology is large and encompasses many different areas. Initially synthetic biology was part of the molecular microbiology field and encompassed the manufacture of small, short lengths of DNA bases to form probes for polymerase chain reactions and antibody fluorescent probes. The use of oligonucleotides has become more widespread in the last 10 to 15 years and they are now used in newer technologies such as microarrays, fingerprinting and Loop-Mediated Isothermal Amplification (LAMP) technology (Mori and Notomi, 2009). These techniques are used as tools during studies that cover areas as diverse as gene expression studies in animals and the study of foetal development to pathogen detection in the environment.

Whilst understanding the function of genes is very important to medical research and developing our existing knowledge, synthetic biology is attempting to take this further. More is now known about genome sequences and the available library of gene functions is constantly increasing. Synthetic biology takes the next step by either modifying genes to create useful products that can be utilised by humans or creating new organisms with sequences that have never before existed in nature. This emerging field has vast resources at it's disposal, with governments and venture capitalists funding the research. Many synthetic biology companies now have a vast portfolio of patents for products or sequences produced by them (ETC, 2007). The first genome of a living organism (*Haemophilus influenzae*) was published in July 1995 (Fleischmann *et al*, 1995) and this marked a change in the advances of biotechnology. The discovery of restriction enzymes by Arber, Nathans and Smith in 1978 (Roberts, 2005) allowed existing organisms to be modified into producing useful products for humans such as inexpensive drugs and fossil fuel alternatives. The industry has helped to generate products that are difficult to produce naturally.

The benefits of synthetic biology can be divided into two categories, focussing on advancing and developing existing knowledge and creating new products (Forster and Church, 2007). Because synthetic biology is a developing technology, there are few examples of its application at present (RAE, 2009), but there are some from USA. For example Artemisia, the sweet wormwood plant which provides the precursor for an anti-Malaria drug, is notoriously difficult to propagate and only grows in specific parts of the world. A synthetic biology project however by Prof. Jay Keasling and co-workers at University of California Berkeley has succeeded in engineering yeast to produce artemisinic acid, the precursor for artemisinin, an anti-malarial drug (Ro *et al*, 2006). Another example is work by Dr Chris Voigt and co-workers at University of California San Francisco on engineering *Salmonella typhimurium* to secrete spider silk proteins, with potential use as light and extremely strong woven material.

Beyond this there are a number of proof of concept studies based on developing technologies to make the engineering of biology more straightforward and reliable. These include work at Boston University to produce an engineered genetic toggle switch in *E. coli* to become light sensitive and express fluorescent protein to produce a photographic 'lawn' of bacteria.

The table below, from the RAE report (2009), lists the major topics currently being explored for synthetic biology applications.

Health	Energy	Environment	Agriculture	Other industry
Cell counter Biological sensors Disease diagnosis Disease fighting Controlling signs of ageing Custom drugs Tissue engineering	Bio power units Biofuels Enzymes Artificial leaf	Emissions sensors Spill/chemical/radiation detection Biodegradable packaging Stronger/lighter materials	Starch synthesis New seed products Bioenergy feedstock Agro-fuels Optimised food production	Biological computers Digital/bio converters Logic gates Switches/oscillators Cleansing biofilms Responsive materials, e.g., oil Nano particle production Bioremediation Biofabrication

The following table, also with data taken from the RAE report (2009), lists companies currently known to be active in synthetic biology.

Company	Subject area
Amyris Biotechnologies, California USA	Drug Development and Biofuels
Blue Heron, Washington USA	Gene synthesis
Chromatin Inc, Illinois USA	Agriculture
DNA2.0, California USA	Gene synthesis
Genscript, New Jersey USA	Pharmaceuticals and biotechnology
Gevo, Colorado USA	Biofuels
Greenfuel Technologies Corporation, Massachusetts USA	Biofuels
LS9, California USA	Biofuels
Mascoma Corporation, Massachusetts USA	Agriculture and energy
New England BioLabs, Massachusetts USA	Production and supply of reagents for the life science
Scarab Genomics, Wisconsin USA	Clean genome *E. coli*
Synthetic Genomics, California USA	Energy and environment

Company	Subject area
BP, Global	Biofuels
Bioneer, South Korea	DNA Purification
DSM, The Netherlands	General
GENEART, Germany	Gene synthesis
Genencor, Denmark	Agriculture and food
ProtoLife, Italy	Modelling technology

Synthetic biology also has close links to engineering disciplines, and we are seeing more and more collaboration between the genetics and engineering fields of science, for example at the International Genetically Engineered Machine Competition (iGEM; http://ung.igem.org/Main_Page) which was first started in 2003 and is held every year globally. In this competition people from both disciplines are encouraged to work together to produce new applications for synthetic biology. The participants (usually undergraduate students) are given a kit at the start of the summer from the Registry of Standard Biological Parts, from which they have to use alongside parts of their own design to produce new biological systems, which can operate inside living cells. This competition has grown rapidly from a meagre five teams in the first year to 130 in 2010. Projects range from a rainbow of pigmented bacteria to an arsenic sensor to banana smelling bacteria. Whilst the finished projects may sometimes be humorous, there is a serious element to this competition as it brings together biology and engineering, and encourages innovation by scientists who may in future assist in the development of the potential applications of synthetic biology described above.

4.2 SYNTHETIC BIOLOGY IN BRITAIN

A number of British universities are engaged in synthetic biology as a goal or as a means to an end, i.e., used to study something else. There are also a few British commercial companies that manufacture short sequences of DNA probes, e.g., Alta Biosciences; or the design and manufacture DNA vaccines, for example Oxford Biomedica and Novartis. A detailed list of institutions currently involved in synthetic biology will be discussed in an accompanying report (HSL Report HEX/10/11).

5 SYNTHETIC BIOLOGY: RISK VERSUS BENEFIT

5.1 PUBLIC PERCEPTION OF THE TECHNOLOGY AND ETHICAL IMPLICATIONS

In theory, synthetic biology can offer many benefits to society. However every new technology, particularly one that alters the fundamental code of life, has disadvantages as well as benefits. As this field of science is relatively new, the ethical arguments for and against can be discussed early and proactively.

The ethical debate surrounding synthetic biology is extensive and ongoing, as reviewed in detail by Balmer and Martin (2008). The main questions focuses on whether it is acceptable to manufacture modified organisms that would not have evolved naturally, and as such this ethical debate is linked to religious views. Critics of synthetic biology consider that the scientists involved are 'playing God' with nature. The term 'life' is defined in many ways, and can be interpreted differently according to which view you take. Scientists involved in synthetic biology use the term life literally to mean *'the condition which distinguishes active organisms from inorganic matter including the capacity for growth, functional activity and continual change preceding death'* (EU, 2010). This views a living organism as a finely tuned machine capable of performing a number of processes that differentiate it from inorganic matter, such as metabolism, homeostasis, and the ability to grow, reproduce and evolve through the process of natural selection. By contrast, there is the notion of living things as factories for processes. Opponents support the belief that it is inappropriate to define a human life in this way, because it dismisses the fact that life is also an expression of our social and cultural existence, that deserves care and respect and which centres on the concept of human dignity.

There are three main ethical viewpoints relevant in the discussion of synthetic biology. The first is the Eco-centric view, where the preservation of nature is the main focus point. Secondly, the anthropocentric view which suggest that humans are the most important species on earth and that nature can and should be manipulated for human purposes. And finally there is the biocentric view that extends inherent value to non-human species, ecosystems and processes in nature. These three value systems all have different focus points. The anthropocentric viewpoint clashes with the Eco-centric view that the environment should be protected and should not be manipulated for human use (EU, 2010).

A worry for the general population is the risk of an escaping organism becoming a threat to our environment, ecosystem or culture. Ecosystems are very finely tuned, and there are several examples of human introduction of existing species into different ecosystems that have had major impacts. The addition of modified bacteria could have huge implications, including higher up the food chain. Schmidt *et al* (2009) suggested that if proper regulation and risk assessments are put in place to prevent malicious use, the likelihood of an untoward incident involving accidental or deliberate release of a modified organism could be significantly reduced. The management and regulation of modified organisms through risk assessments is therefore a priority.

Another concern is the regeneration of previously existing pathogens. For example, scientists have been able to recreate the particularly virulent influenza strain which caused the devastating Spanish Flu pandemic of 1918, and also the virus which causes polio, a highly contagious and debilitating disease which has been eliminated in the Western population thanks to a rigorous programme of vaccination which started in the 1960s (Cello *et al*, 2002). Critics have opposed the creation of previously eradicated pathogenic species calling it irresponsible. However it is difficult to know where to draw the line. If the research into pathogens doesn't continue, then

possible cures and vaccinations won't be discovered in the event of a deliberate release of a virulent bacteria or virus. If such an event were to occur, questions would be asked as to why the research into the pathogenic organisms wasn't made available. There is a fine line that has been drawn, as to how many groups would benefit from advances in synthetic biology as a whole.

The potential for synthetic biology has far reaching consequences on the world. A good example of this is the cultivation of Artemisia, the precursor to an anti-malaria drug. Cultivation of this particular crop has become a lucrative business, and a good opportunity for poorer farmers to make more money selling this sought after plant. Critics have warned that the ease of producing Artemisic acid synthetically will negatively impact on these poorer communities, as there will no longer be any need to grow this crop if there is a cheaper alternative available. However Wellhausen and Mukunda (2009) conducted a detailed study into effects of synthetic biology on third world economies, using the production of rubber in Malaysia and Indigo dyes in India as case studies. They found that replacing natural products with synthetic products was not as detrimental as first thought and that it did not automatically lead to the extinction of the natural product.

Closely linked to ethical implications is the problem of censorship, i.e., should research related to synthetic biology be made public? With the emergence of DIY biology (see below), there is a responsibility by the scientists conducting research to ensure that their methods do not get into the wrong hands. For example the method for developing the poliovirus (Cello *et al*, 2002) was published alongside the paper, so anybody with knowledge of molecular biology could recreate the virus, with the right equipment, discretely in a laboratory built at home. This raises the chances of this or any other pathogen being weaponised by terrorist organisations. However if methods like this aren't published, scientists involved in legitimate research won't be able to use their methods, and the laws of censorship could also be called into disrepute.

Some scientists within the synthetic biology community have suggested this can be done by self-regulation (ETC, 2007), where the people involved in the research could develop risk assessments relating to their individual studies. However, civil society organisations rejected these plans, instead suggesting that the society as a whole should have a say on it. It has also been suggested that synthetic biology cannot be regulated solely on a national level, and a global context has to be taken into account. The feasibility of a global regulatory body is debatable.

The role the general public play in the risk assessment and publication of new technologies should not be underestimated. Their perception of the technology, influenced by the media and peer-pressure, has been seen to limit research and the end-use of a technology. This is particularly evident in the subject of genetic engineering, with particular reference to the modification of organisms destined to enter the food system. The negative public perception of genetically modified foods significantly limited progress in this area of science, but lessons have been learned on how to change the general perception of modifying organism for human benefit, including increasing honesty and openness to ensure the trust of the public.

On the whole, evidence suggests (RAE, 2009) that the public sees synthetic biology in a more positive light than genetically modified foods, even though in essence they are one and the same. The idea behind this is if the research is open and available to the public, and the positive benefits are clearly presented, and they are seen to be of general benefit rather than providing profit for commercial companies, the negative media and public image of GM foods will be avoided.

One interesting point to note is that the public seems to be more averse to modifying plants and crops that will be eventually sold on the supermarket shelves, than they do to modifying organisms to for medical purposes or to create bio-fuels. The reason for this concern may be to do with the idea that gene transfer can happen more readily in the natural environment, than it can in a laboratory, because fertilisation of crops is a natural and uncontrollable process, performed by the wind and insects. In a natural environment, wild-type plants can reproduce with the pollen of genetically modified plants to create plants that could proliferate at a high rate and swamp any variation in the ecological population. The general public are also more averse to eating what they term as 'Frankenstein-foods', as the culture for organic, naturally produced and environmentally friendly food has become more popular.

Rabinow and Bennett (2009) has described the attention to issues of safety, and social consequences of synthetic biology as ''safety-by-design.'' Safety-by-design is an attempt to extend self-governance models developed in the early days of genetic modification, such as at the 1974 Asilomar conference. Examples include efforts to design in genomes that maximise control over their function, such as minimising the risk of survival or re-programmability outside of the laboratory. The purpose of safety-by-design is to account for, and prepare for, both negative and positive effects in advance (Garfinkel *et al*, 2007; Church, 2005). However, it could be compromised by security issues arising from dual-use (see below) which is the misuse of technologies created for benevolent purposes.

5.2 PERCEIVED RISK OF TERRORISM AND 'DUAL USE'

As with any emerging fields of science, there is the risk, not wholly unjustified, that the new technology might be used to cause harm to people or the environment, especially in the hands of terror organisations. This issue is closely linked to the public perception of synthetic biology, the idea that new 'super-bugs' or highly contagious viruses could be introduced into the population via a contaminated water or air supply.

In a report produced by the Biotechnology and Biological Sciences Research Council in collaboration with the Engineering and Physical Sciences Research Council (2009) that centred around dialogue with the public on the issues related to synthetic biology, many of the participants raised concerns that other countries might not have as strict regulations as the Britain in controlling the use of synthetic biology and believed that it would be difficult to create global standards. Furthermore, the emergence of 'DIY' or 'garage' biology would allow security measures to be bypassed, to allow terrorists to produce particularly virulent strains of bacteria, viruses or toxins. However, current evidence would suggest that bio-hackers are just curious biologists, not intent on creating malicious pathogens, but instead focussing on more novel ideas.

In a report commissioned by the European Union (EU, 2010), concerns were raised over bio-terrorism and whether future life science discoveries could be open to 'dual-use' with implications for developing bioweapons, even going so far as to suggest that a publishing ban should be considered. Two examples were cited; the genetic engineering of vaccine-resistant mousepox and the artificial synthesis of the polio virus. It has been argued that publication of this work could alert would-be bio-terrorists to possibilities, as well as providing explicit instructions for producing biological weapons. Conversely, publishing work potentially yields benefits for medicine and the opportunity to share ideas, while issues related to the freedom of science and censorship emerge.

The cost and analytical sophistication for DNA synthesis means there are relatively few companies undertaking it, and it was reported that these companies could screen all sequences

for toxicity or infectivity before processing an order. However, that would only work if databases of toxic or infective DNA sequences are available, and if copyright protection does not restrict access to the information. The existence of software termed 'BlackWatch' was cited as being available from CRAIC (2010) for the purpose of tracking DNA sequence synthesis which may be hazardous. The software being developed would be able to address the 15 million orders a month worldwide that are expected by 2012 (DOTS, 2010). The mechanism for operating such a system, including cost, and who to and how to report potentially harmful sequences, would need to be established.

5.3 POTENTIAL FOR GARAGE SYNTHETIC BIOLOGY OR DIY BIOLOGY

Outside of the formal regulated laboratory there is an area of synthetic biology activity called 'DIYBio' or 'garage biology'. There are some established networks in USA, but it is difficult to determine the extent of activity in the Britain. Contacts with British synthetic biology laboratory research groups have suggested there is some activity, and this has been summarised in the report on work activities in Britain (HSL Report HEX/10/11). Summarised below are some of the potential risks that may arise from DIYBio.

A review performed by the ETC group (ETC, 2007) states that the cost of DNA synthesis is decreasing rapidly as the processes become more routine and the technology more advanced. They reviewed the cost of producing one base pair, and found that in 2006, the average price (in US dollars) for most gene synthesis companies was $1-2, with the cheapest company charging $0.85 per base pair. At a synthetic biology conference in 2006, most gene synthesis companies were predicting that the prices would drop to $0.50 by the end of 2007. The turnround time in which these companies can produce genes is also decreasing. Taking as an example the virus phiX 174 which has a 5,386 bp genome, this could now be synthesised for approximately $6000 and take less than a month to manufacture.

These developments make it easier for the typical 'garage biologist' to obtain DNA sequences quicker and easier than ever before. As the field of DIYBio becomes increasingly progressive and pervasive, regulation becomes more difficult. There are also a growing number of chemical supply companies on the internet. These companies operate world-wide and may not be subject to the same health and safety regulation as companies based in Britain. In our view, regulation is essential at the source, i.e., restrictions and guidance should be given to the companies producing the genes to stop people buying them for hostile uses and the possibility of a world-wide regulatory body to manage these companies is feasible but fraught with difficulties. The Black Watch software as described above is already in use by a number of companies in the genetic industry, that allows the company to scan incoming orders for known pathogenic sequences, and question and stop the production of genes that could be potentially harmful to a human population if inserted into a vector (bacteria, fungi or virus) and transmitted into the air or through a water supply.

Schmidt (2008; *this paper also gives links to hacker sites*), asserts that there exists a kind of informal code of ethics for the biohacker community that demands to ''be safe, do not damage anything, do not damage anyone, either physically, mentally or emotionally". The biohacker community have recognised not only the risk to their own health but the negative publicity and there are moves towards addressing safety issues, for example biohacker sites offering advice on ''how to use a pressure-cooker as an autoclave'', and providing links to laboratory safety videos. However, this code is voluntary and unless a major environmental release occurred it is hard to perceive how non-compliance could be identified, let alone regulated even though in theory GM regulations (see below) apply as they apply to the individual.

6 THE CURRENT REGULATORY POSITION AND RISK ASSESSMENT PROCEDURES

6.1 THE CURRENT SITUATION IN BRITAIN

As synthetic biology develops, robust risk assessments will be needed for occupational health protection and to address public health concerns. At present, risk assessments for synthetic biology work contained within the laboratory are covered by Genetically Modified Organisms regulations, in Britain this being the Genetically Modified Organisms (Contained Use) Regulations 2000, which implement the European GMM Contained Use Directive (2009/41/EC). This provides a framework for risk assessment, notification and permissioning in laboratories undertaking GM work and therefore also synthetic biology activities. Other aspects of synthetic biology research, such as use of chemical agents, would be covered by COSHH. Subject to a current review of biological agents regulation, the Genetically Modified Organisms (Contained Use) Regulations 2000 may be subsumed into the Biological Agents and Genetically Modified Organisms (Contained Use) Regulations 2012 (http://www.hse.gov.uk/biosafety/callaghan.htm), but the GM regulatory framework would remain largely unchanged.

With the increasing momentum towards development of synthetic agents, as started by Craig Venter's work to create new organisms, new risk assessment methods and regulations will have to be considered to include detailed analysis of the function of the organism, and the potential consequence of accidental or deliberate release. As US and British research centres are in the forefront of synthetic biology research, it would be expected that they would be heavily involved in development of new regulations if needed, or examining whether current regulations are fit for purpose.

6.2 USA REGULATIONS

Laboratory regulation in USA is currently the remit of the National Institute for Public Health (NIH) "The NIH Guidelines for Research Involving Recombinant DNA Molecules" (http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf).

Their system for categorisation of biological agents is fundamentally the same as the European and British system, using BioSafety Levels (BSL) 1 to 4, with 4 being the most hazardous to human health. Laboratory design and containment, as in Britain, is proportionate to biohazard and the work being done. Researchers are required to perform a risk assessment on the work they are doing, taking into account factors including the host organism, the virulence, pathogenicity, route of spread and stability. The NIH Guidelines are currently being revised to more explicitly address synthetic nucleic acids (Patterson, 2010).

A US Presidential Bioethics Commission conference in July 2010 (http://www.bioethics.gov/transcripts/synthetic-biology/) on synthetic biology addressed a broad range of regulatory and ethical topics as a general overview, while a limited number of reports have considered the current and future regulatory needs in more detail. Recent released voluntary framework guidance by the US Department of Health and Human Services is the 'Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA'. The aim of this is to minimise the risk that unauthorised individuals or individuals with malicious intent will obtain "toxins and agents of concern" through the use of nucleic acid synthesis

technologies by encouraging record keeping of supply of materials etc. http://www.phe.gov/Preparedness/legal/guidance/syndna/Pages/default.aspx.

The US National Science Advisory Board for Biosecurity (NSABB) has produced a number of reports on synthetic biology, the most recent in April 2010 providing a useful overview of the current position (NSABB, 2010), including the uncertainty of risk assessment. In this, they make the following recommendations:

1. Synthetic biology should be subject to institutional review and oversight since some aspects of this field pose biosecurity risks.

2. Oversight of dual use research should extend beyond the boundaries of life sciences and academia.

3. Outreach and education strategies should be developed that address dual use research issues and engage the research communities that are most likely to undertake work under the umbrella of synthetic biology.

4. The US Government should include advances in synthetic biology and understanding of virulence/pathogenicity in efforts to monitor new scientific findings and technologies, such as "tech-watch" or "science-watch" endeavours.

6.3 EUROPEAN COMMISSION (EU OPINION 25 REPORT) - LEGAL, GOVERNANCE AND POLICY ASPECTS

A recent report by the European Commission (EU, 2010) reviewed the status of regulatory frameworks covering synthetic biology internationally. It stated that while specific legislation on synthetic biology has not been introduced in European Union Member States, the technology is covered by existing regulations which transpose EU legislation into national legal systems. There are further global provisions issued by the World Trade Organisation (WTO), and an international framework on ethics and human rights, although the latter is legally binding only to a limited extent.

At the EU level, the covering legislation is that for GMOs, biomedicine, bio-safety, chemicals, data protection and patents. Global provisions on these are issued by WTO and bio-safety standards are issued by the World Health Organisation (WHO), further supplemented by international frameworks on ethics and human rights. At present, most approaches to synthetic biology involve the use of genetic modification techniques, therefore within the EU they are regulated through GMO Directives. These are Council Directive 2009/41/EC on the contained use of genetically modified micro-organisms, Directive 2001/18/EC on the deliberate release into the environment, Regulation (EC) No 1946/2003 on transboundary movements of genetically modified organisms that implemented the provisions of the Cartagena Protocol on Biosafety within the European Union. Most of the work in synthetic biology falls within the remit of Directive 2009/41/EC which deals with the contained use of genetically modified micro-organisms. Legislation related to the placing of products on the EU market, e.g., medical devices, medicinal products and cosmetics, is harmonised at Member State level, whereas for Good Clinical Practice Community law establishes minimum provisions, supplemented by national legislation. Data protection and patent provisions are set at EU Member State level, with WTO agreements forming the legal provisions for international commerce. The international framework on ethics and human rights is legally binding only to a limited extent. The Council of Europe Convention on Bioethics (1997), based on the Convention for the Protection of Human Rights and Fundamental Freedom (4.11.1950), is binding for the States

that have signed and ratified it, but not all EU countries have done so. However, European funded research projects have to comply with the principles enshrined in the Bioethics Convention. Whilst also not legally binding, UNESCO Declarations and the EU Charter of Fundamental Rights provide moral authority. All of the above may be supplemented by national regulations. However, there may be limitations. The Nuffield Council (2009) acknowledged that the above EU regulatory frameworks address the biosafety of synthetic biology, but risk assessments made under GMO regulations compare the altered organism with the natural organism on which it is based, considering the individual traits introduced. Synthetic biology could produce organisms with multiple traits from potentially several different donor organisms and the current biosafety framework may not provide sufficient reliability to the risk assessment and analysis framework.

In addition to the requirements identified above, there may be further requirements depending on the use to which the products of synthetic biology might be put, including:

- New medicinal products (Regulation (EC) No 726/2004, Directive 2001/83/EC, Directive 2003/94/EC and Directive 2003/63/EC);
- Medical devices (Directive 93/42/EEC and 90/385/EEC);
- Gene therapy, cell therapy and tissue engineering (Regulation (EC) No 1394/2007 amending Directive 2001/83/EC and Regulation (EC) No 726/2004, Directive 2001/83/EC, Directive 2004/23/EC and Directive 2002/98/EC);
- Clinical trials (EC 2001/20 amended in 2003 and 2005;
- Cosmetic products (Directive 1976/768/EC);
- Data protection (Directive on the processing of personal data and the protection of privacy in the electronic communications sector;
- Chemicals (REACH rules);
- Biological risks (Council Directive 82/894/EEC and Council Directive 2000/29/EC of 8 May 2000; and
- Safety and health for workers exposed to biological agents at work (Directive 2000/54/EC).

Further regulatory frameworks at the EU and international level that may also apply include patenting, open access and those covering (CBRN) biosecurity.

The EU report (2010) also described frameworks at an international level. WHO biosafety standards set out in their Laboratory Bio-safety Manual (2004) encourages countries to accept and implement basic concepts in biological safety and to develop national codes of practice for the safe handling of pathogenic microorganisms in laboratories within their geographical borders. The manual stresses the importance of personal responsibility and addresses risk assessment, safe use of recombinant DNA technology and transport of infectious materials, biosecurity by protection of microbiological assets from inappropriate use. In 2000, the Convention on Biological Diversity adopted a supplementary agreement to the Convention known as the Cartagena Protocol on Biosafety. (Convention on Biological Diversity, 2010) The Protocol seeks to protect biological diversity from the potential risks posed by living modified organisms resulting from modern biotechnology. It established a procedure for ensuring that countries are provided with the information and documentation necessary to make informed

decisions before agreeing to the import of modified organisms into their territory, and established a Biosafety Clearing House to facilitate the exchange of information on living modified organisms. The EU and all EU Member States have ratified the protocol under Regulation (EC) No 1946/2003 on transboundary movements of genetically modified organisms.

World Trade Organisation (WTO) agreements and Trade-Related Aspects of Intellectual Property Rights (TRIPS) provide governance models that should address several dimensions of synthetic biology policy and activities, including:

- Monitoring of research and safety issues;
- Monitoring ethical criteria to be implemented for synthetic biology research;
- International legislation or regulation to clarify legal grey areas;
- Professional level self-regulation and codes of conduct;
- Scientific level expectations from scientific results, priority setting, resource allocation;
- Institutional level risk assessments and implementation of risk management;
- Societal level protection of citizens' rights and liberties.

Below the legislative level, at the 'soft law' level, governance models have been proposed by the Industry Association for Synthetic Biology (2008) to cover actions arising from production, distribution and registration of potentially dangerous DNA sequences. Similar options for governance have been proposed by the J. Craig Venter Institute (Garfinkel *et al*, 2007). However, such self regulation by those involved in synthetic biology research raises questions about legitimacy, credibility and public trust (ETC, 2007), although it would be a sensible approach for researchers to assist in developing such codes that could then be implemented and monitor by public authorities. Additional questions relate to the role the public should play in governance of synthetic biology. An editorial in Nature suggested that self-governance does not preclude other forms of governance (Nature, 2006).

Assessment and evaluation of risks associated with synthetic biology raises a number of concerns. For example in contained use:

- How to assess the safety of organisms that have a genome derived using recombinant DNA techniques and that allow the production of systems combining elements from multiple sources;
- How to evaluate the biological safety of constructions in organisms that may contain genes or proteins that have never existed together in a biological organism or that contain newly designed biological functions that do not exist in nature;

And for (planned or inadvertent) release to the environment:

- Unknown risks to the environment and public health through unexpected interactions between synthetic microorganisms and the environment or other organisms in it;

- Horizontal gene transfer and the potential impact on ecosystems;
- The interaction of synthetic micro-organisms with naturally-occurring substances; or
- Unforeseen evolution of synthetic biology agents.

In concluding that "biosafety considerations are pre-requisites for the promotion and implementation of an EU synthetic biology research program, both nationally and internationally", the EU Group also concurred with the Nuffield report, in that assessment methods for GMOs are based on a comparison of the altered organism with the natural organisms on which they are based, considering each individual trait introduced. However, synthetic biology could result in organisms with multiple traits from multiple organisms, making it difficult to predict their properties. The outcome of this, they reported, is that some scientists have proposed that in absence of clear biosafety data all synthetic biology research protocols should take place at Biosafety (Containment) Level 3 or 4. This would have major implications for the development of this scientific sector. The Group therefore recommended that the European Commission should initiate a study on current risk assessment procedures in the EU to survey relevant biosafety procedures and identify possible gaps in the current biosafety regulation to effectively assess organisms and novel products developed through synthetic biology, also indicating the mechanisms to fill the identified gaps. They proposed that risk assessment procedures identified should be administered by Competent Authorities within the EU and be conditional for financing of synthetic biology research and the marketing of synthetic biology products in the EU. At an international level these biosafety rules should be used to facilitate a standardised approach to biosafety of synthetic biology for public and private funded trials and the establishment of instruments to monitor the implementation of such provisions. Furthermore, the Group advocated that a Code of Conduct for research on synthetic micro-organisms should be prepared by the European Commission to ensure that synthetic biology organisms are manufactured in a way that they cannot autonomously survive in case of accidental release into the environment.

6.4 NETHERLANDS COGEM REPORT - WILL THE RISK ANALYSIS FOR GMOS SUFFICE FOR SYNTHETIC ORGANISMS?

At a national level, possibly the most comprehensive assessment of their legislative position has been published by the Netherlands legislative body for GMOs COGEM (2008). In this report, they acknowledged that some scientists have reservations that some human and environmental risks may be hard to assess or may simply not be identified at all, while others consider that the risks are not so great because synthetic organisms will be built according to a predetermined plan.

COGEM considered three criteria to judge whether developments in synthetic biology would affect human and environmental safety:

1. Legislation: is there a legal framework for action?
2. Risk management: can technical safety measures be taken to manage risks?
3. Risk analysis: can the risks be assessed?

Regarding legislation, they considered that the Dutch Environmental Management Act, the Genetically Modified Organisms Decree (Environmentally Hazardous Substances Act) and the

Ministerial Regulation on GMOs, translating EU Directives into national law, also apply to synthetic organisms. They reasoned that because fundamentally the techniques used to create synthetic organisms are the same as those used for GMOs, synthetic organisms therefore fall within the legislative framework for GMOs and new legislation governing synthetic biology is not necessary.

Regarding risk management, GMO regulations were also considered applicable to synthetic organisms. The regulations guarantee the safety of humans and the environment during laboratory work with both GMOs and wild type micro-organisms by providing measures to prevent organisms from being released from the laboratory to the outside environment or infecting laboratory workers, including for example regulations on laboratory design and equipment, working practices, use of personal protection, disinfection and waste treatment. The COGEM report also described their precautionary principle, by which if it is not possible to assess the risks (to a sufficient degree) because of scientific uncertainties, activities involving GMOs are assigned to a higher containment level than might be strictly necessary. This precautionary principle also dictates that new technologies may not be used without taking precautionary measures if they are likely to involve risks to human or environmental health, even if those risks have not (yet) been established without doubt by scientific research. All the above was considered to apply not only to GMOs, but also to synthetic organisms. However, it was acknowledged that a high level of containment could incur significant costs and organisational difficulties, such that placing activities in too high a containment category could hinder scientific and commercial progress.

Regarding risk analysis methodology, COGEM considered the focus on:

- The properties of the GMO and of the vector and donor sequences of the parental wild type;
- The exposure of humans and the environment;
- The nature of any negative effects caused by the GMO or the parental wild type; and
- The probability that these effects will occur.

To assess risks, consideration is given to the biological containment and pathogenicity of the donor and recipient organism, the vector used and the presence of a characterised or uncharacterised insert, also the activities involving the GMO. Having considered all of these, a statement can be made about the risks of the activities in question. The greater the difference between the parental organism and the modified organism, the more difficult it is to compare the characteristics of both organisms, thus limiting the predictive value of the risk analysis.

This is important for synthetic biology. Although in general the current risk analysis system for GMOs can also be used for the manufacture of synthetic organisms and activities involving them, difficulties arise in assessing the characteristics of an organism that has been created via the bottom-up approach, for example, if an organism has been created to which various new metabolic pathways have been added. Once again, although the precautionary principle could be applied, this may present an unnecessary impediment to scientific and commercial progress.

As technological developments are also resulting in greater understanding of genes and the possible interactions between gene products, COGEM considered that the assessment of risks may become easier. As a result, as the technology develops the possibilities for assessing risks

will usually improve thereby meaning that risks associated with future activities will usually become more predictable over time. In line with a step-by-step approach used for GMOs, this means that the first experiments are carried out on a small scale in the laboratory until sufficient data have been obtained. Once these data are available, the activities can be carried out on progressively larger scales with more data becoming available at each subsequent step.

In the short term, it was considered that researchers will generally only work with known apathogenic or low-pathogenic organisms such as the well established apathogenic *Escherichia coli* K12, or *Saccharomyces cerevisiae* (baker's yeast), as used to produce artemisinine. Also in the short term it was considered that only biologically contained organisms would be used, with characteristics that restrict their survival or dispersal in the environment. These could include 'minimal genome organisms' that are biologically contained, given that they only possess the most essential genes and can replicate only in special culture media and under specific conditions, and as such would have gene functions that would be known in almost all cases. They predicted that it would be unlikely within the next ten years that hereditary material would be introduced into an organism without knowledge of its function or a predetermined plan, especially as knowledge of the genome and the characteristics of an organism would be indispensable to obtain a functional organism. Metabolic pathway engineering was cited as an example. Without knowledge about the genes to be introduced there would be little chance of creating a functioning pathway. 'Biobricks' were also cited as an advantage because they are well defined pieces of hereditary material therefore their functions are fully known, while knowledge of the introduced hereditary material was considered essential for the synthesis of a minimal genome organism via the bottom-up approach of inserting genes according to a predetermined plan.

An exception to the above was cited in which random insertion of a large number of genes from various sources into an organism is done all at the same time, a method that closely resembles so-called 'shotgun' experiment in genetic modification to manufacture a GMO in which sequences are used that consist entirely or partly of non-characterised genetic information.[22] Such shotgun experiments are used to make gene banks. Although the resulting organisms are mostly less fit than the parental organism, theoretically a more harmful organism could be created, and a lack of knowledge about the introduced genetic material makes risks harder to assess, therefore greater safety measures are needed than with experiments using fully characterised hereditary material. For example, under the precautionary principle experiments in which many genes are inserted into synthetic organisms in a random manner would need to be done in a high-level containment laboratory.

It was predicted that over the next five to ten years work on synthetic organisms would remain restricted to laboratories and production facilities where potential risks can be controlled. In the longer term, experimentation is likely to include metabolic pathway engineering, and an example was given of the production of a precursor of artemisinine by genetically modified *E. coli* bacteria and *S. cerevisiae* yeast cells (Martin *et al*, 2003; Ro *et al*, 2006). To produce artemisinic acid in *S. cerevisiae* requires increasing or reducing the expression of certain genes in the yeast cell as well as introducing several genes from *E. coli* and the plant *Artemisia annua* (the natural producer of artemisinine). The introduced pathway consists of twelve genes in total. In this example only a limited number of known genes are introduced into the organism which is biologically contained and thus potential risks can be adequately assessed. Also, the function of the introduced gene makes it unlikely that biological containment would be overcome. In future, however, pathways consisting of hundreds or even thousands of genes from various sources could be built into organisms, making risk assessment more difficult because of natural modifications and complex interactions. For example, a risk might arise from the unintentional production of a toxic metabolite due to interference between an introduced pathway and an existing pathway. Conversely, it was predicted that in future

technical development there would be more possibility of detecting and predicting changes in metabolites and unintentional effects of interactions between them.

Potential risks from minimal genome organisms were considered. For example, the bacterium *M. genitalium* has been reduced to its minimum number of protein coding genes to survive in culture medium, 100 of 482 proving non-essential (Glass *et al*, 2006). This was top-down technology, involving a host whose complete genome sequence is known, and once a minimal genome organism has been created, hereditary material can be added to give it the desired function, in much the way the researchers at the Craig Venter Institute created a synthetic *Mycosplasma* species earlier in 2010 (Gibson *et al*, 2010). The alternative, to create a minimal genome organism via the bottom-up approach, is only likely to succeed by following a predetermined plan because the chance of obtaining a functioning organism through the random assemblage of genes or DNA fragments is very small. This would require knowledge of the host and the genes or biobricks to be introduced, and therefore the ability to carry out a risk analysis.

Novel genetic material was considered, for example, a set of two new nucleotides has been developed that can be recognised by a natural polymerase (Leconte *et al*, 2008). Attempts to create unnatural nucleic acids consisting of different backbone molecules have included the use of novel informational biopolymers such as: Threose Nucleic Acid (TNA), Glycol Nucleic Acid (GNA), Hexitol Nucleic Acid (HNA), Locked Nucleic Acid (LNA), or Peptide Nucleic Acid (PNA):. (Chaput *et al*, 2003, Zhang *et al*, 2005, Vandermeeren *et al*, 2000, Ng and Bergstrom 2005, Schoning *et al*, 2000, Kaur 2006, Orgel 2000, Vester and Wengel 2004). In future, these could replace existing nucleotides and already more than 30 unnatural amino acids have been added to proteins in various organisms (Xie and Schultz, 2006), but only in non-living systems. It was considered that this technology (so called 'alternative alphabet') is at such an early stage that there is the opportunity to assess potential risks over the long term. Also, as the altered building blocks do not exist in nature, the organism would have to take them from the surrounding environment making them totally dependent on specific culture conditions in a laboratory for replication and/or protein synthesis. These systems are therefore biologically contained and the risks can be assessed. A theoretical next step would be to engineer a replicating organism capable of producing unnatural nucleotides and passing them on to future generations but if this could be done at all it would be several years away.

The ultimate aim would be to develop synthetic organisms that could be introduced into the environment, for example to target and break down toxic contaminants. This would necessitate a risk assessment including full molecular characterisation of the organism and the inserted genes, as well as knowledge of the environment into which the organism would be introduced and any possible interactions between the organism and the ecosystem. It would also be necessary to know whether the organism would be restricted to the place in which it was introduced or whether it could disperse more widely.

6.5 STEPS TOWARDS ASSESSING RISKS ASSOCIATED WITH PROTOCELLS (BEDAU, 2009)

Regarding the development of protocells, six key stages listed as checkpoints have been proposed that represent major step changes in the technology which have significant ethical, social, or regulatory implications (Bedau, 2009, Rasmussen *et al*, 2009). These are:

- Checkpoint A: Advancing research in protocell synthesis should trigger consideration of ethical, social and regulatory implications. The authors considered that this checkpoint has already been reached, and discussion has already begun.

- Checkpoint B: The technical feasibility of autonomous protocells being surmounted. This is a major social and ethical checkpoint that precedes the actual existence of protocells.

- Checkpoint C: Creating the first fully autonomous protocell in the laboratory would involve creating a self assembling and self-reproducing chemical system with the properties of containment, metabolism, and programmability. This is the single scientific protocell achievement with the greatest social and ethical significance.

- Checkpoint D: Protocells able to survive outside the laboratory. These would have the potential to cause harm to human health and the environment, so should trigger a re-assessment of regulation and containment standards.

- Checkpoint E: Release of protocells outside the laboratory (possibly for commercial reasons). This would have special social significance because protocells would be in direct contact with the broad range of life forms, including humans.

- Checkpoint F: Protocells that are toxic or infectious would trigger the need for appropriate safety regulations.

If toxic or infectious protocells were created but contained in the laboratory (Checkpoint C) that would already be a risk. That risk would greatly increase if protocells were used outside the laboratory (Checkpoint E) in medical or environmental applications, because their use would depend on their proliferation. The conclusion of the authors was that regulatory bodies are already reviewing this technology while it is still at the developmental stage, and not yet technically within reach. However, they proposed that regulators should review whether protocell research and development, and the future existence of protocells, would reveal gaps in the current regulatory structures. This review needs to be in place before Checkpoint B (technical feasibility). They recommended that this should include ensuring that regulators are equipped with the necessary knowledge to apply their mandate to the technology. Arguably, this review project for HSE is fulfilling this recommendation.

In comparing protocell development to the current systems that classify biological agents into four biosafety levels, Bedau (2009) recommended that an analogous classification system should be developed for working with protocells in the laboratory. It was speculated that some properties of protocells could be predicted now, while some would be recognised only after protocells were developed that would be capable of surviving outside the laboratory, or when toxic or infectious protocells were possible, i.e., when Checkpoints D and F are reached. Upon creation of the first fully autonomous protocell and thereafter, protocell safety classification would need to be re-examined and revised regularly because it is only as those checkpoints are reached that scientists would understand many underlying mechanisms of protocells and the associated safety issues. Protocells will be able to take up and metabolise material from the environment, reproduce, and evolve. The ability to evolve could potentially cause problems for human health or the environment and appropriate safety mechanisms would be needed. These could include so-called “dependable’’ systems in computer science and engineering with resilient, built-in safeguards.

6.6 BIOSAFETY CONSIDERATIONS BY INTERNATIONAL ASSOCIATION FOR SYNTHETIC BIOLOGY (IASB)

IASB published a report on the outcome of a workshop - Technical solutions for biosecurity in synthetic biology (IASB, 2008). In it, it was acknowledged that the perceived risks with synthetic biology were no different from general molecular biology and genetic engineering at present, but that new biosafety risks could emerge from synthetic biology in a relatively short time. Main areas of public concern were uncontrolled environmental release and creating artificial life, therefore it was seen as important that lessons were learnt from the early stages of recombinant DNA technology, in which it was considered mistakes were made. Examples cited of the way forward were ensuring the scientific community plays a leading role in addressing risks and ethical issues and introducing pre-emptive policy initiatives, better engagement with the public by stimulating open public debate, and they advocated applying tight regulation in the beginning which can be relaxed over time. It was also seen to be important to develop coordinated regulation for biorisks, biosafety and biosecurity.

6.7 VIEWS FROM MARKUS SCHMIDT AT THE ORGANISATION FOR INTERNATIONAL DIALOGUE AND CONFLICT MANAGEMENT BIOSAFETY WORKING GROUP, VIENNA, AUSTRIA (SCHMIDT 2008; 2009; 2010; SCHMIDT ET AL, 2009)

Markus Schmidt is probably the most widely published author on synthetic biology and biosafety, and his experience in the field has been used by the iGEM competition to construct their biosafety web page information and guidance for the 2010 competition. (http://2010.igem.org/Safety). He describes synthetic biology as an interdisciplinary field, involving chemists, biologists, engineers, physicists and computer scientists. The disadvantage is that some of those scientists are generally educated in disciplines that do not routinely include formal biosafety training. He recommends (see also Garfinkel *et al*, 2007) that, to overcome the possible problems of new researchers with a professional background other than biology and who are unskilled in the handling of (dangerous) biological material in the laboratory, there should be moves to:

- Include biosafety training as part of an interdisciplinary education in synthetic biology, dealing with risks and best practices as part of college and university curricula, critical for at least priming these newcomers to the safety challenges in synthetic biology;

- Prepare a biosafety manual for synthetic biology laboratories, distinct from those manuals already available;

- Broaden the review function of Institutional Biosafety Committees (IBC) to include enhanced oversight and/or enforcement.

He conceded however that these strategies would be practically useless if the newcomers were not working in a professional setting and were not accountable to a public authority, such as biohackers.

In addressing the safety requirements for standardised bioparts, Schmidt promoted the idea of a toolbox of bioparts that could be easily assembled to devices and systems. This concept would not only greatly simplify the design process of living organisms. As the Biobrick registry (http://partsregistry.org/Main_Page) develops, and more people have general access to sequence specifications and DNA synthesis, it was considered that the task of enforcement by restricted access or practice would be increasingly untenable (see also Carlson, 2007), but on the other

hand the fully detailed characterisation of parts accessed through this scheme would be an advantage from a risk assessment viewpoint. Biosafety concerns could be raised because emergent behaviour of novel biocircuits could not be ruled out due to the lack of sufficient separation of functional units (such as in integrated circuits) and potential number of interactions between those units. Schmidt (2008) calculated that, in theory, a relatively small number of 20 bioparts may result in up to 20x19x18x17…x2x1 or about 10^{18} possible interactions. This would make it difficult to calculate all interactions and likewise hard to rule out the possibility of emergent behaviour.

Other suggestions included:

- The need to think about safety standards when dealing with parts, as some parts could be more of a safety problem than others, thus leading to a requirement for different safety categories for different parts, also for devices and systems;

- Combinations of otherwise safe parts could result in gene circuits with unsafe characteristics, therefore a need to include safety checks in bio-circuit design;

- In future some chassis will need to be able to survive in the soil, e.g., for bioremediation purposes and these would need to be treated differently from chassis that can only survive under certain laboratory conditions. Further safety categorisation would be needed for parts, devices and systems that could extend the environmental range of a chassis, for example to tolerate a wider range of biotic and abiotic conditions;

- The development of a Biosafety clearinghouse, so that if an unforeseen (emergent) safety issue was discovered in a certain bio-circuit other people could learn from the experience;

- Integration of safety and security aspects into the design process so that design software automatically informs the designer of the potential for newly designed circuits to exhibit safety (or security) problems; and

- Whether a new risk assessment tool is needed to ensure safety (and security) of parts-based biocircuits.

Several new challenges arise from such systems. If it is assumed that a biological system has been designed and inserted into a host (or chassis), short term questions include:

- Can behavioural characteristics of the new network be predicted to a degree of certainty that allows a reasonable estimation of risk factors?

- What happens to the network if one or several parts change their function or stop working as intended? How will the whole network change its characteristics?

- How can the genetic/functional robustness be measured? What would be a meaningful and suitable “unit” for robustness in bio-circuits? Do different forms of applications require different levels of robustness (for example, cells in an industrial fermenter vs cells in human body, e.g., for insulin control)?

- How reliable is the biological circuit? How can reliability be measured and what are meaningful units?

- Could there be an unplanned event or series of events resulting in death, injury, occupational illness, damage to or loss of equipment or property, or damage to the environment?

- Could biocircuits be designed to avoid crosstalk between functional elements of its circuit?

In the longer term, the following questions could also arise:

- How to deal with new biocircuits that involve deliberately engineered complex behaviours such as non-linearity, path dependent behaviour, randomisation, or chaotic characteristics.

- Will it be possible to program a cell that can reprogram itself?

Schmidt (2009) stated that the datasheets on registered Biobrick parts have little information on safety, only including a description of the reliability of simple parts, distinguishing genetic reliability and performance reliability, such as the number of generations it takes to cripple 50% of the circuits in the cells (Canton *et al*, 2008). Although this is a useful starting point, more information would be needed for a proper risk assessment process to decide whether a biocircuit is safe enough for commercialization or release into the environment.

In considering minimal genomes (see also COGEM summary in Section 6.3) Schmidt (2009) acknowledged that by definition they would be restricted to a very narrow ecological niche, thus being safe organisms that could only inhabit particular environments and will not be able to exist outside of these. However, he considered it valuable for trials to be undertaken with minimal cells in environments that differ from their original optimal environments in order to generate real experimental data on the range of suitable environments for the minimal organism. This would allow for better predictions of their real environmental host range. Other experiments would include proof of the inability of the minimal organism to survive anywhere else than under defined laboratory conditions, and establishing how long it would take (if at all) under perfect laboratory conditions for the minimal organism to evolve to a non-minimal organism, for example through horizontal gene-flow from other organisms. Further evaluation would be necessary after minimal organisms have had novel biological circuits (such as parts, devices, systems) implanted, because these "synthetic organisms" could no longer be considered as minimal organisms. Consideration would be needed as to whether the implanted biological circuits could enlarge the environmental niche of the cell.

Regarding protocells (see also Section 6.4) created from bottom up synthesis, such cells could show some but not all of the characteristics of life (compartmentalisation, growth, metabolism, evolution, reproduction, replication, autopoesis, response to stimuli) but are likely to be largely disabled. It is possible that the first protocells would be mandatory symbionts to natural forms of life in order to survive. If so, the host range would need to be identified to avoid unlikely but not impossible "infections" by protocells, especially if they are very different from natural cells.

Regarding the use of novel genetic materials (alternative alphabets), Schmidt (2010) and Schmidt *et al* (2009) acknowledged that it is unlikely for synthetic organisms to be created in this way in the foreseeable future, but that if it could happen the question would arise as to how to assess the potential risk such organisms could present, for example a novel type of virus was generated based on a different nucleic acid and using an unnatural reverse transcriptase, or an organism based on an enlarged genetic alphabet that could avoid natural predators at all, enabling almost unrestricted spread.

Schmidt argues in favour of biosafety engineering, both in his 2009 publications and in the advice contained in the iGEM biosafety web pages, describing synthetic biology as changing biotechnology into a computable, controllable and predictable engineering discipline, also using an alternative description of "intentional biology". Thus synthetic biology could be the ultimate biosafety tool because it can avoid unintended consequences, but this assumes an ability to control all biological processes in an engineered system. Safety engineering, he argues, is already an established subset of systems engineering in disciplines such as mechanical engineering, aviation, space flight, electronics and software. Professional knowledge and skills are applied to scientific and engineering principles, criteria, and techniques to identify and eliminate hazards and thus to reduce associated risks. Safety engineering assures that a system behaves as needed even when parts of it fail.

For synthetic biology to be assessed in the same way, it can apply principles of safety engineering, such as how to design a fault-tolerant system, a fail-safe system or ideally an inherently safe system. Fault-tolerant systems will continue to operate with nonfunctional parts even if with reduced performance. Some level of redundancy included in systems increases robustness against random failure of parts. Where this analogy is limited however, is where self-replication is feasible, adding further complication. Biosafety engineering could be used to design genetic circuits that would still work or would not cause harm to human health or the environment on failure.

Techniques used in safety engineering include inductive approaches (Event Tree Analysis) and deductive approaches (Fault Tree Analysis) (NASA, 2002, NUREG, 1991), both used in safety assessment in aircraft, space travel, mechanical engineering and nuclear energy. They employ the use of standard parts and engineering designs. The inductive approach assesses any event in a system and its effect on the whole system. In molecular biology, that could be a mutation of a genetic part that makes it dysfunctional. The Event Tree Analysis (ETA) would examine how the whole system would be affected by this failure, in terms of whether the system could still fulfil its tasks, whether it would cause it to behave in a different way or shut down completely. This analysis would dictate whether additional safety systems would be needed, such as redundant sub-circuits.

The Fault Tree Analysis technique (FTA) examines defined unwanted failures in a system then traces back to the causes. For example, it would be an unwanted feature for a genetic circuit to fail in a way that leads to overproduction of a particular protein normally regulated. FTA would show which events could cause overproduction and be used to improve the circuit to avoid this unwanted failure, for example by designing a circuit so that failure events would cause the expression of the protein to diminish but never to increase.

For synthetic organisms potentially being released to the environment, ETA and the FTA could be used to design less robust organisms by designing an in-built weakness to ensure that the organism could survive outside its designated target environment. Another example would be to control organisms by incorporating basic metabolic pathways that require essential biochemicals incapable of being synthesised by the organism therefore having to be supplied externally (auxotrophy).

6.8 AN ASSESSMENT FROM THE ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

A report by OECD and the Royal Society (2010) described the potential values of synthetic biology and the regulatory framework required. It was estimated that around £17 million ($27

million) has been allocated to synthetic biology projects and related activities, mainly through government funding with partners such as the Wellcome Trust. An important feature of this is the development of a common language between scientific and engineering disciplines, and their involvement of social science including ethics and risk assessment.

In this report, the regulatory implications of synthetic biology were considered to be of immediate concerns to the British government, it being stated that the official view is that most synthetic biology research will be covered by current regulations on genetically modified organisms (GMOs) and that there is no need, at present, for new regulations relating specifically to synthetic biology. However it was noted that, because it is not possible to clearly define new technology or draw boundaries around what is included or excluded, it is important to encourage self-regulation as well. Regulations for the safe use of biotechnology and recombinant DNA technology were considered to be robust, supported by data suggesting that no major incidents have been known to occur. However, while synthetic biology is considered an extension of these technologies, questions need to be asked continually as to whether current regulatory regimes continue to be adequate. Examples of areas identified that may require future examination are as follows:

- The difficulty of identifying pathogenicity for synthetic agents. With conventional organisms or GMOs, taxonomy of the parent organism provides data on pathogenicity, but for novel organisms the lack of prior experience would present a particular challenge. Identifying sequences with pathogenic properties is also difficult, and conventional tools may no longer be appropriate.

- The 1974 Asilomar conference on biosafety was a starting point for the first rDNA regulations from which current GMO regulations globally have evolved. However, it is argued that the changing science and technology, including the world wide web, as well as the political landscape, means that technology is increasingly available and easy to access, with greater proliferation and distribution of knowledge. While this is positive, it makes it more difficult to monitor and regulate biosafety. The broader range of disciplines being used in synthetic biology may help progress, but could lead to differences in biosafety awareness and training, while concern was raised about security threats outside the laboratory.

- It was felt that there might be a need to revisit established concepts in biosafety and biosecurity, to re-define the definitions or ‘harm’ and the ‘natural environment’ in terms of regulation for technical needs and to allay public anxiety.

Within the OECD report, four risk factors for synthetic biology were outlined, being the technologies themselves, the practitioners of these technologies, the biology and the public. Synthetic biology was divided into two types of technologies, being genome synthesis and engineering. Both present risks, because with genome synthesis it might be possible to build *de novo* an organism which can escape current system controls, while engineering techniques include molecular shuffling or self-replicating systems which could also threaten biosafety. It was considered that integration of the following actions would lead to better evaluation of risk and a regulatory framework suitable for synthetic biology:

- Develop uniform and standardised screening tools to evaluate risk, especially for synthetic genomics;

- Develop a rationalised list of agents to determine the most dangerous or risky and prioritise screening. It was acknowledged that this would be difficult given that the

- Build a database of risky sequences or experiments to help stratify and keep track of risk.

7 RECOMMENDATIONS AND IMPLICATIONS

A number of papers and reviews on synthetic biology over the last five years have described how the technology will change the world. Theoretical uses for synthetic biology are broad, ranging from making new bio-fuels, cleaning up environmental pollutants, creating micro-processors for computers, to creating biosensors that can be injected into hospital patients and administer drugs to the precise site of illness as required. These applications signify a new era in biology, although in reality most work is still at the theoretical stage. It is therefore important, before significant levels of practical work start, that a robust risk assessment process and a proportionate regulatory framework are in place to provide worker protection and public confidence.

The process by which a gene is inserted into an organism uses well-known molecular biology techniques. Traditionally, this work has been performed in university or commercial research laboratories but, although still costly, some reduction in sequencing costs has raised fears that synthetic biology could become less regulated if undertaken by individuals outside the laboratory. However, the main focus will continue to be on laboratory safety and containment.

Regulation of laboratories with regard to biosafety varies from country to country but most work with a similar regulatory framework in which GMO legislation is applicable to synthetic biology. In the EU, European Directives, which also follow the WHO guidelines, are applied via national legislation, while in USA the national legislation is administered by the National Institute for Public Health. In all cases, the main focus of the regulations is that of a risk centred approach. Any work performed should have an appropriate risk assessment that takes into account the host organism, the type of genes being introduced to the host and any potential increase in virulence or pathogenicity.

Within the large body of literature dealing with synthetic biology, some dealt with biosafety and ethical issues but only a smaller proportion dealt with biosafety and occupational and environmental risk assessment. However, as reviewed in this report, some detailed evaluations are available, for example describing the regulatory framework in Europe and how that has been applied. Recommendations with regard to the approach to risk assessment and controls are consistent with those currently applied to GMOs, but a number of reports and papers also highlighted the future challenges that may arise from the ‘bottom-up’ (protocell) approach to synthetic biology design, where an organism is not being modified but created, also the unpredictable elements of novel genetic material (alternative alphabet). Some reports also raised as a possible concern the potential handling of synthetic organisms by non-biologists, with some recommendations as to how that should be addressed.

In summary, the current regulatory framework for GMOs in Britain adequately covers present and near future synthetic biology activities, but it will be important to maintain a watching brief on new developments in technology so that HSE, as the regulatory body, will be equal to the challenge of reviewing project notifications and associated risk assessments. This review has provided details of current approaches to biosafety risk assessment and the potential future challenges that might require additional guidance or more detailed independent assessment from the regulator. Using the precautionary principle, to dictate that work with synthetic agents is only conducted in high containment facilities, could be considered disproportionate. However, proportionate control will rely on researchers being able to provide robust and clearly argued risk assessments. It will be important for regulators and dutyholders to develop and maintain a dialogue to ensure that this procedure is put in place.

8 REFERENCES

Balmer A, Martin P (2008). Synthetic biology ethical and social challenges. BBSRC Report May 2008. Available at http://www.bbsrc.ac.uk/web/FILES/Reviews/0806_synthetic_biology.pdf#search=%22balmer%22

BBSRC (2009) Synthetic Biology Dialogue Prepared by: Bhattachary D, Pascall Calitz J, Hunter A. Available at http://www.bbsrc.ac.uk/web/FILES/Reviews/1006-synthetic-biology-dialogue.pdf#search=%22synthetic%22

Bedau MA Parke EC Tangen U Hantsche-Tangen B (2009). Social and ethical checkpoints for bottom-up synthetic biology, or protocells. Syst Synth Biol 3:65–75

Canton B, Labno A, Endy D (2008) Refinement and Standardization of Synthetic Biological Parts and Devices, Nature Biotech, 26(7):787–793

Carlson R (2007) Laying the foundations for a bio-economy. Syst Synth Biol 1:109–117. doi:10.1007/s11693-007-9010-z

Cello J, Aniko V et al. (2002). Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. Science 297: 1016-1018

Chan LY, Kosuri S, Endy D. Refactoring bacteriophage T7. Mol Syst Biol. 2005; 1. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1681472/pdf/msb4100025.pdf

Chaput JC, Ichida JK, Szostak JW (2003) DNA Polymerase-Mediated DNA Synthesis on a TNATemplate, J Am Chem Soc, 125, 856–857

Church G (2005) Let us go forth and safely multiply. Nature 438:423.

COGEM. Biological machines? Anticipating developments in synthetic biology COGEM Report 2008; CGM/080925-01

Convention on Biological Diversity. Cartagena Protocol. Website accessed at http://bch.cbd.int/protocol/

CRAIC Blackwatch. Details available at https://biotech.craic.com/blackwatch/introduction.html

DOTS - DNA Order Tracking System. Details available at: http://www.mitre.org/news/digest/advanced_research/02_09/genes.html

ETC (2007). Extreme Genetic Engineering: An Introduction to Synthetic Biology January 2007. Available at www.etcgroup.org

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

Fleischmann RD, Adams MD, White O, Clayton RA, et al (1995). Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science 269; 496-512

Forster AC, Church GM (2007). Synthetic biology projects in vitro Genome Res. 17: 1-6.

Garfinkel M, Endy D, Epstein G, Freidman R (2007) Synthetic genomics: options for governance. Biosecur Bioterror 4:359–362;

Gibson DG Glass JI, Lartigue C, Noskov VN, Chuang R-Y et al. (2010) Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome *Science* **329**, 52.

Glass JI, Assad-Garcia N, Alperovich N *et al.* (2006). Essential genes of a minimal bacterium. PNAS 103(2): 425-430

Heinemann M, Panke S (2006). Synthetic biology -putting engineering into biology. Bioinformatics 22: 2790-9.

Holley RW, Apgar J, Everett GA, Madison JT, Marquisee M, Merrill SH, Penswick JR, Zamir A. Structure of a ribonucleic acid. Science. 1965; 147: 1462-5

IASB. Technical solutions for biosecurity in synthetic biology **(**2008) Report on the workshop held on April 3rd, 2008 in Munich. Compiled by Bernauer H, Christopher J, Deininger W, Fischer M, Habermeier P, Heumann K, Maurer S, Schwer H, Stähler P, Wagner T. Available at www.ia-sb.eu

Jones S, Thornton JM. Principals of protein-protein interaction. Available at http://www.pnas.org/content/93/1/13.full.pdf

Kaur H, Arora A, Wengel J, Maiti S (2006) Thermodynamic, Counterion, and Hydration Effects for the Incorporation of Locked Nucleic Acid Nucleotides into DNA Duplexes, Biochemistry, 45 (23):7347–7355, doi:10.1021/bi060307w.

Kaznessis YN. Models for synthetic biology. BMC Syst Biol. 2007; 1: 47.

Keasling JD (2008). Synthetic biology for synthetic chemistry. ACS Chemical Biology 3: 64-76.

Leconte AM, Hwang GT, Matsuda S *et al.* (2008). Discovery, characterization and optimisation of an unnatural base pair for expansion of the genetic alphabet. J Am Chem Soc 130: 2336-2343.

Ledford H (2010). Life hackers. Nature, 467: 650-652.

Lehn JM. (2002) Toward self-organization and complex matter. Science 295: 2400-3.

Luisi PL, Ferri F, Stano P. (2006). Approaches to semi-synthetic minimal cells: a review. Naturwissenschaften; 93: 1-13.

Martin VJJ, Pitera DJ *et al.* (2003). Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nature Biotechnology 21(7): 796-802

Mori Y, Notomi T (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother. 15:62-69.

NASA (2002) Probabilistic Risk Assessment Procedures Guide for NASA Managers and Practitioners, Available via: http://www.hq.nasa.gov/office/codeq/doctree/praguide.pdf

Nature Editorial (2006). Policing ourselves. Nature 441: 383

Neumann H, Neumann-Staubitz P (2010). Synthetic biology approaches in drug discovery and pharmaceutical biotechnology. Appl Microbiol Biotechnol 87:75–86

Ng PS, Bergstrom DE (2005) Alternative Nucleic Acid Analogues for Programmable Assembly: Hybridization of LNA to PNA, Nano Lett, 5(1): 107–111

NSABB (2010). Addressing biosecurity concerns related to synthetic biology. Draft Report of the National Science Advisory Board for Biosecurity (NSABB) Available at: http://oba.od.nih.gov/biosecurity/pdf/NSABB%20SynBio%20DRAFT%20Report-FINAL%20(2)_5-21-10.pdf

Nuffield Council on Bioethics (2009). Forward Look Seminar April 2009; Note of meeting topic 4 synthetic biology. Available at http://www.nuffieldbioethics.org/sites/default/files/files/Forward%20Look%20note%202009.pdf

NUREG 0492 (1991) Fault Tree Handbook – Reliability and Risk Analysis, Norman JMcCormick, Academic Press, New York

OECD (2010) Symposium on Opportunities and Challenges in the Emerging Field of Synthetic Biology Synthesis Report OECD, Royal Society. Available at: www.oecd.org/sti/biotechnology

Orgel L (2000) A Simpler Nucleic Acid, Science, 290(5495):1306–1307.

Parliamentary Office of Science and Technollogy (2008). Postnote 298; Synthetic Biology. Available at: http://www.parliament.uk/documents/post/postpn298.pdf

Patterson AP (2010). Federal oversight of synthetic biology research. Presentation at US Presidential Bioethics Commission conference, July 2010. Available at: http://www.bioethics.gov/transcripts/synthetic-biology/

Rabinow P Bennett G (2009). Synthetic biology: ethical ramifications. Syst Synth Biol 3:99–108

RAE (2009). Synthetic Biology: scope, applications and implications The Royal Academy of Engineering ISBN: 1-903496-44-6. Available at: www.raeng.org.uk/synbio

RAE (2009). Synthetic Biology: public dialogue on synthetic biology. The Royal Academy of Engineering. Compiled by King S, Webster T. ISBN: 1-903496-46-2 June 2009. Available at: www.raeng.org.uk

Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) (2009) Protocells: bridging nonliving and living matter. MIT Press, Cambridge

Ro D-K, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, Ho KA, Eachus RA, Ham TS, Kirby J, Chang MCY, Withers ST, Shiba Y, Sarpong R and Keasling JD (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440: 940-943.

Roberts RJ (2005). How restriction enzymes became the workhorses of molecular biology. Proc Nat Acad Sci 102; 5905-5908. Available at http://www.pnas.org/content/102/17/5905.full

Schmidt M (2008) Diffusion of Synthetic Biology: A Challenge to Biosafety. Systems and Synthetic Biology, 2(1–2):1–6

Schmidt M (2009). Do I Understand What I Can Create? Biosafety Issues in Synthetic Biology In M. Schmidt et al. (eds.), *Synthetic Biology*, DOI 10.1007/978-90-481-2678-1_6,

Schmidt M (2010) Xenobiology: A new form of life as the ultimate biosafety tool. BioEssays 32:322–331.

Schmidt M Ganguli-Mitra A Torgersen H Kelle A, Deplazes A, Biller-Andorno N (2009). A priority paper for the societal and ethical aspects of synthetic biology Syst Synth Biol 3:3–7 DOI 10.1007/s11693-009-9034-7

Schoning K, Scholz P, Guntha S, Wu X, Krishnamurthy R, Eschenmoser A (2000) Chemical Etiology of Nucleic Acid Structure: The Alpha-Threofuranosyl-(3'–>2') Oligonucleotide System, Science, doi:10.1126/science.290.5495.1347.

Szostak JW, Bartel DP, Luisi PL (2001). Synthesizing life. Nature 409: 387-90.

Vandermeeren M, Préveral S, Janssens S, Geysen J, Saison-Behmoaras E, Van Aerschot A, Herdewijn P (2000) Biological Activity of Hexitol Nucleic Acids Targeted at Ha-ras and Intracellular Adhesion Molecule-1 mRNA, Biochem Pharmacol, 59:655–663

Vester B, Wengel J (2004) LNA (Locked Nucleic Acid): High-Affinity Targeting of Complementary RNA and DNA, Biochemistry, 43 (42), 13,233–13,241

Wellhausen R Mukunda G (2009). Aspects of the political economy of development and synthetic biology. Syst Synth Biol 3:115–123

WHO Laboratory Biosafety Manual 3rd Edition (2004). Available at http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Xie J, Schultz PG (2006). A chemical toolkit for proteins – an expanded genetic code. Nat Rev Mol Cell Biol 7: 775-782

Zhang L, Peritz A, Meggers E (2005) A Simple Glycol Nucleic Acid, J Am Chem Soc, 127: 4174–4175

Published by the Health and Safety Executive 09/12

HSE

Health and Safety Executive

Synthetic biology

A review of the technology, and current and future needs from the regulatory framework in Great Britain

Synthetic biology has been described as ‘the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes’. It encompasses engineering of DNA-based biological circuits using standard biological parts, finding the minimal genome capable of functioning, constructing protocells, ie, living cells from scratch, and chemical synthetic biology in which biological systems are created based on a biochemistry not invented by evolution (ie, not the G-C-A-T nucleic acid backbone). It has developed from genetic technology that has discovered the functions of genes and proteins to enable the above developments, assisted by supporting technology that now allows gene sequencing to be done more quickly and at a much lower cost, as well as the emergence of commercial sequencing services and standardised production of genetic sequences, or ‘Biobricks’. The consequence of this development of the technology is to make it more widely accessible and expand it beyond microbiology into the disciplines of engineering, chemistry and computing.

This report and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

RR944

www.hse.gov.uk