Workshop on "Synthetic biology: containment and release of engineered micro-organisms"

held on 29 April 2013

at King's College London

SUMMARY OF DISCUSSIONS

Claire Marris and Catherine Jefferson

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Note that this document is a summary of discussions that occurred at the workshop and that the authors do not necessarily endorse the positions reported. Arguments are reported even when they were only expressed by one or a few participants. The aim of this document is to represent the diversity of views expressed, and does not seek to assign particular weight to the arguments reported. This document does not represent a consensus position.

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This report, as well as the Scoping Report prepared for this workshop and the slides from the presentations, can be downloaded from the SSHM website: http://www.kcl.ac.uk/sshm

or are available by e-mailing claire.marris@kcl.ac.uk

Comments are welcome and should be sent to claire.marris@kcl.ac.uk

Executive Summary

Many of the envisaged applications of synthetic biology, especially in the environmental and agricultural sectors, would involve the use of engineered micro-organisms outside of laboratories and industrial installations. The aim of this workshop was to explore the prospects for these synthetic biology applications, including issues related to regulatory appraisal, environmental risk assessment, and risk reduction measures proposed by synthetic biologists. The key themes that emerged from the workshop discussions are summarised below.

The need to understand the whole system
In the 1980s and early 1990s there was much talk and hope about using genetically modified micro-organisms (GMMOs) for agricultural and environmental applications, yet that first wave of enthusiasm and research did not lead to commercially viable products. Microbiologists who had been involved in field experiments with genetically modified micro-organisms (GMMOs) in the 1990s noted that a key reason why that avenue of research had been discontinued was that soil ecosystems were diverse, complex and poorly understood. This meant that engineering micro-organisms that could reliably perform their intended function once released into the environment was very difficult. Participants agreed that synthetic biology would need to draw on expertise in ecology and soil microbiology not only to understand potential impacts and to inform the assessment of risk, but also in order to help improve the viability of products proposed for deliberate release into the environment.

Survive or die?
The discussions illustrated that there is a balance to be reached between making GMMOs survive long enough in the environment to perform their intended function, and aiming to contain the organisms and the inserted or modified genetic material in order to reduce the potential for environmental harm. There was a general agreement that most of the attention in risk research, risk assessment and policy discussions surrounding environmental risks has focused on how to make sure that the genetically engineered organisms released into the environment (or other organisms that pick up their genetic material) do not survive and 'take over' natural ecosystems, i.e. 'the superbug question'. Some participants suggested that this might be the wrong question and that the real challenge is how to create micro-organisms that could compete well enough with pre-existing bacterial populations in order to perform reliably and effectively in the environment. This was essential for the GMMOs to deliver their intended benefits and/or to be of commercial interest.

Blurred boundaries
Participants discussed the blurred boundaries between 'contained use' and 'deliberate release' of GMMOs. Some participants noted the importance of the context of use in determining whether a product is considered 'contained' or 'released into the environment'. For example, a trained scientist using a live bacterial biosensor contained in a vial and used in a 'lab in a van' in Germany is very different from a non scientist using the same product in a village in Bangladesh. Participants also discussed the difference between product-based and process-based regulations and their implications for how genetically modified organisms are treated differently by US and EU/UK regulatory systems. Regulators and others noted that there are blurred boundaries around these categories, which have to be constantly re-examined. For some participants, this appeared somewhat illogical or inconsistent, especially when different regulatory authorities (e.g. US/EU) make different judgements about the same 'things'.

Engineered biocontainment
There was general agreement that engineered biocontainment based on built-in genetic designs could never be fail-safe: they may be able to decrease, but could not completely eliminate, the frequency of Horizontal Gene Transfer (HGT) and the likelihood of persistence and dispersal of released micro-organisms. Bacterial populations have well-known mechanisms, including HGT, that can enable them to evolve and circumvent biosafety designs. Therefore, given high enough population numbers and/or long enough timescales, bacteria may override any kind of 'kill switch'. Participants had different opinions about the implications this has for the role of engineered biocontainment for biosafety. For some participants, the focus should be on trying to improve genetic biocontainment designs and to better quantify the incidence of HGT using mathematical models and/or field experiments. Others

suggested the focus should shift towards research to evaluate the potential consequences on human health and the environment if and when HGT occurred and/or the GMMO did persist or disperse following the deliberate release of a specific GMMO into the environment. Some synthetic biologists thought that engineered biocontainment could, in circumstances where the potential negative environmental impact of HGT or dispersal was assessed as low, be considered an appropriate approach to help maximise biosafety. Other participants (including NGOs, microbiologists and regulators) stressed that engineered biocontainment is not by itself an adequate biosafety approach. Regulators present emphasised that the key question for risk assessment was to assess whether or not the GMMO released could be associated with potential harm, and that HGT in itself does not constitute a hazard.

Risk, benefit and uncertainty

Different approaches to risk were apparent in the discussions. Some participants stressed that risk is a narrow concept, and is radically different from uncertainty. They argued that a risk-based approach leads to focusing attention on measuring and modelling probabilities of known adverse events, ensuring that identified risks are 'low', and developing ways to contain hazardous materials. While this is the current dominant approach and seemed suitable to many participants, others contested its appropriateness and argued that past lessons called for more precautionary and participative approaches that would build on a wider range of expertise to help open up areas of uncertainty, ambiguity and ignorance. This would focus on unanticipated as well as known adverse effects. The importance of assessing benefits as well as risks was also stressed by many participants. Some participants felt that this could help accelerate regulatory decisions for applications that were thought to have obvious humanitarian benefits, while others suggested that, if conducted methodically, benefit assessment could help challenge unwarranted claims about benefits and open up deliberations about their nature (e.g. Who benefits, and under what conditions? How does this solution compare with other options to address the same problem?).

Effects of over-promising

Several participants suggested that the current focus on demonstrating impact in grant applications can lead to exaggerated claims about the speed and scope of future economic and social impact of research projects. A number of participants suggested that such over-promising could have undesirable effects. Some cautioned that this could lead to disenchantment if promises are not met, which could be damaging to the field. Some suggested that because synthetic biology is seen as a new and promising field, it is attracting substantial funding and young researchers, which could lead to other fields such as microbiology or ecology being under-resourced.

The need to understand 'the problem'

Several participants stressed the importance of comprehensively understanding the nature of the problem that synthetic biology applications are proposing solutions to. A number of participants argued that synthetic biology is a 'technology looking for a problem', i.e., it is starting with the tools and looking for ways to apply them, rather than looking at a problem and finding ways to solve it. This was considered by these participants to be detrimental because it did not encourage deliberation about choices between different approaches to tackle a problem, and also because it tended to be associated with simplistic understandings of complex problems that neglected the entanglement of biological, social, economic and political dimensions involved. Synthetic biologists argued that this was an unfair criticism of the field and that it was not unique to synthetic biology.

Responsibility for 'broader questions'

Synthetic biologists present generally agreed that there were real and important 'broader questions' about the purpose of their work, but they disagreed about the extent to which they could or should be held responsible for addressing all these questions. Some argued that dealing with them was outside their remit or sphere of expertise; whereas others stated that scientists involved in the research should be involved in addressing these questions. Social scientists and NGOs present did not expect laboratory scientists to be held responsible for all the impacts of their work, and focused instead on the responsibility of synthetic biologists who are promoting the field to temper their claims and to acknowledge uncertainty. Some also argued that responsibility for identifying what counts as a problem and determining the best approach to deal with it needs to be shared more broadly.

Summary of Discussions

Many of the envisaged applications of synthetic biology, especially in the environmental and agricultural sectors, would involve the use of engineered micro-organisms outside of laboratories and industrial installations. The aim of this workshop was to explore the prospects for these synthetic biology applications, including issues related to regulatory appraisal, environmental risk assessment, and risk reduction measures proposed by synthetic biologists. The workshop brought together a range of participants from different sectors of society with different kinds of expertise, different perspectives on synthetic biology and also, significantly, different perspectives on the relationship between 'science' and 'society'. The workshop brought together synthetic biologists, microbial ecologists, regulators, public research funders, experts from environmental and food safety NGOs, and social scientists with expertise in governance and environmental risk assessment. (These categories were not mutually exclusive: for example, some synthetic biologists had been trained as microbiologists, and several social scientists, NGO representatives, and regulators had PhDs in microbiology or molecular biology). In order to facilitate open and productive discussion, a scoping report was prepared and circulated to all participants in advance of the workshop and the meeting was held under the Chatham House Rule.

Note that this document is a summary of discussions that occurred at the workshop and that the authors do not necessarily endorse the positions reported. Arguments are reported even when they were only expressed by one or a few participants. The aim of this document is to represent the diversity of views expressed, and does not seek to assign particular weight to the arguments reported. This document does not represent a consensus position.

SESSION 1: Learning from past experiences with released GMMOs

In the 1980s and early 1990s there was much talk and hope about using genetically modified micro-organisms (GMMOs) for agricultural and environmental applications, yet that first wave of enthusiasm and research did not lead to commercially viable products. This session aimed to learn from past experience by drawing on the expertise of two microbiologists who were involved in field experiments at the time. It examined some of the reasons why this first wave of GMMOs failed to realise its promise and explored the prospects for synthetic biology in this area today.

Presentation by Dr Penny Hirsch
Agroecology, Rothamsted Research
"GM *Rhizobium* inoculants as biofertilisers"[1]

Soil micro-organisms perform a number of important functions, particularly in nutrient cycles that underpin soil fertility and plant health. Microbial applications have been used to improve agriculture and horticulture for a long time. For example, *Bacillus thuringiensis* has been used as a biopesticide and *Rhizobium* as a biofertiliser since the 19th century. Modifying root-nodulating bacteria such as rhizobia for improved nitrogen fixation was one of the first targets of genetic engineering investigated in the 1980s. However, while this worked in the lab, results in the field were not consistent due to the variability of soil ecosystems and environmental conditions between sites. Soil microorganisms are very numerous and diverse, only a small minority can be cultured in the lab, and many belong to novel groups. Soil microbiology is heterogeneous, complex and poorly understood, and this is the environment in which GMMOs would be released.

Rhizobia, like other bacteria, are known to have many mechanisms through which they can exchange genes. This is important as the genes controlling symbiosis with plants are located on plasmids in many rhizobia; consequently it is a leaky system, not a contained system. A series of field experiments to assess GM rhizobia as agricultural inoculants were funded by the European Commission from 1996 to 2001, with a number of trials conducted by Penny Hirsch at Rothamsted Research. The aim was to monitor persistence and dispersal of GM rhizobia, and to investigate occurrences of gene transfer. The results challenged some prior assumptions. For example, it was found that introduced rhizobia persist in the field even in the absence of their plant host. It was also found that in the absence of any plants an introduced GM strain which lacked a "symbiotic" plasmid had a survival advantage. Experiments in Germany tested a DNA recombinase deficient ($RecA^-$) strain of *Sinorhizobium* that was expected to be more susceptible to DNA damage from UV light, and hence less likely to persist in the environment, but the results revealed that this did not have any effect on survival of the GM strain.

Overall, the results showed that soil and climatic factors influenced survival of the rhizobia, that gene behaviour was affected by environmental factors, and that improved nitrogen fixation was not reproducible across different environments. Plasmid transfer from GM rhizobia to the natural rhizobia populations was not detected over the 15 year time scale of the experiment, using the techniques available at the time. This does not, however, rule out the occurrence of HGT as it might be detectable over a longer period or using other techniques.

Ultimately funding dried up for these kinds of experiment. This was due to a number of factors including a shift in interest towards the modification of plants rather than bacteria; the increasingly costly process of obtaining approvals for field experiments; and increasing public resistance to GM. However, the main reason for the decline in interest was the inconsistent agronomic results obtained with GMMOs compared to conventional agrochemicals. The GM strains were unable to demonstrate reliable efficacy across different fields. If a 10% yield increase cannot be claimed, agronomists are not interested. The field trials did, however, generate interesting new knowledge about the biology of rhizobium.

One way to overcome the inherent unreliability of GMMOs when used in the environment might be to develop semi-contained applications for which the conditions can be controlled to some extent, for example glasshouse applications or bioremediation treatments for industrial waste stream.

The context for global agriculture has changed since the 1980s. There are now calls for us to move away from a petroleum-based economy towards more sustainable agriculture. Perhaps this will change the way in which GM approaches are considered.

[1] For further details see Hirsch, P. R. (2004). "Release of transgenic bacterial inoculants - rhizobia as a case study." *Plant and Soil*, 266: 1-10.

Presentation by Prof. Ian Thompson

Department of Engineering Science, University of Oxford

"Field release of free-living GM bacteria and prospects for synthetic biology in bioremediation"

The first half of this presentation focused on field releases of free-living bacteria conducted at the University of Oxford Wytham Field Station in 1993-1994. The key research question of interest at that time was persistence of GM bacteria in the environment. To investigate this, the team started by studying the natural bacterial community in the field and then isolated a typical bacteria that they thought would survive well (*Pseudomonas fluorescens*). They then inserted genes into the genome of this bacterium to enable researchers to detect it in samples taken from the field (the GM bacteria could grow on petri dishes containing lactose, xylene and kanamycin). This GM bacterium was released into the field as a seed inoculant where it colonised the roots and leaves of growing crops (sugar beet and wheat), and the survival of the genetically marked bacteria was monitored for two years. People often say that when GMOs are released into the environment they just die, but this was not what was found in this experiment. Results from this field experiment demonstrated that the genetically marked bacteria survived through a season at low but detectable amounts, but not across seasons. Interestingly, they survived longer in the glasshouse than in the environment due to lower competition with other bacteria. The fitness of the bacteria varied with the age of the plant, the season, and the plant material sampled. For example, there were significant differences between bacterial populations recovered from the leaves and roots. Overall, the experiment demonstrated that when the released GM bacteria had to compete with pre-existing bacterial communities in the real world, they survived reasonably well short term but could not compete particularly well in the longer term.

The team also investigated survival of the same bacteria carrying a plasmid which conferred resistance to mercury. Carrying additional genes in the form of this natural plasmid did impact survival, with plasmid-bearing bacteria quickly outcompeted and decreasing to almost undetectable levels in the first 100 days after the initial inoculation. However, once the plant (sugar beet) began to mature, the plasmid seemed to provide a benefit that meant that the bacteria carrying the plasmid reappeared from very low levels to levels equivalent to those of the GM bacteria not containing the plasmid. Overall, the persistence of the released GM bacteria depended very much on the conditions. The experiments also confirmed the complexity of natural soil microbial populations: more than 2000 genotypes of *Pseudomonas* were detected, with only 26 detected more than once. A key conclusion, that the team emphasised when speaking to the Department of Environment who had funded the research, was that the results showed that no single GM bacterium was likely to persist after deliberate release into the environment. What determined the persistence of the overall population of *Pseudomonas fluorescens* was the dominance of particular genotypes that survived best in particular conditions, there was a continual turnover of the population as those conditions changed. This is also an important lesson for those interested in synthetic biology applications in the environment[2].

The second half of this presentation explored the prospects for synthetic biology in bioremediation. Waste clean up is an important challenge for industry. The company co-founded by Ian Thompson, Microbial Solutions, uses non-genetically engineered bacteria to detoxify industrial waste streams. The company does use GMMOs, but only in contained environments, as sensors. Synthetic biology may be able to improve the process of microbial-based detoxification but industry is currently unwilling to use GM organisms. Another reason for not using GM or synthetic biology is that in many cases there are natural microbes available that are already effective: synthetic biology cannot outbid 3.5 billion years of evolution. There is no point in trying to synthesise synthetic bacteria when natural bacteria can already do the job. There may be a role for synthetic biology to address 'pinch-points' where natural bacteria are ineffective. For example, there is a treatment plateau of concentrations of toxic materials under which natural organisms are not effective. Maybe synthetic biology can be used to improve this. But for now it may be best to focus on applications that do not require deliberate release, such as biosensors, the production of pharmaceuticals and bioenergy in bioreactors, or DNA data storage. When a GMMO is released into the environment it is unlikely to compete successfully with indigenous microbial populations. In contained situations, efficacy is easier to manage because there is less competition from other bacteria. In the longer term synthetic biology could help to develop tools for understanding microbial ecology and physiology, which is an area that needs further research.

[2] For further details, see: Lilley, A. and Bailey, I (1997). "Impact of Plasmid pQBR103 Acquisition and Carriage on the Phytosphere Fitness of *Pseudomonas fluorescens* SBW25: Burden and Benefit". *Applied and Environmental Microbiol*ogy, 63(4): 1584-1587.

DISCUSSION

A number of themes emerged from the discussion:

In order to develop viable synthetic biology applications that would require release into the environment to perform their intended function, there is a need to draw on ecological expertise; additionally, synthetic biology could also be a useful research tool to better understand ecosystems.

Both of the talks by microbiologists who had been involved in field experiments with genetically modified micro-organisms (GMMOs) in the 1990s noted that that a key reason why that avenue of research had been discontinued was that soil ecosystems were diverse, complex and poorly understood. This meant that engineering micro-organisms that could reliably perform their intended function once released into the environment was very difficult. Participants agreed that synthetic biology would need to draw on expertise in ecology and soil microbiology not only to understand potential impacts and to inform the assessment of risk, but also in order to help improve the viability of products proposed for deliberate environmental release.

One participant explained that one possible reason why some of the GM rhizobium biofertilisers that were developed failed to work was that researchers had initially started with microbial strains from their laboratory collections, rather than going out into the field to select natural isolates that would be better able to survive and compete. Several microbiologists argued that the failure of early efforts to produce GMMO agricultural applications led to the conclusion that it would be difficult to improve very much on micro-organisms that are naturally occurring.

There was a general agreement that synthetic biology could also be used as a tool to understand ecosystems, but this part of the field is not widely promoted. One participant voiced their concern that instead of being used for this purpose, the field was in danger of being "hijacked" by an application-driven route, and that this was what had already happened with GM techniques.

Some participants argued that synthetic biology is a 'technology looking for a problem', i.e., it is starting with the tools and looking for ways to apply them, rather than looking at a problem and finding ways to solve it.

The perception that synthetic biology was currently largely driven by applications was interlinked with discussions about the importance of understanding the problems being addressed. There was general agreement that synthetic biology offers great potential in terms of techniques and has attracted smart people, but some participants felt that in order to successfully apply those techniques, it is important to ask the right questions and use a broad range of expertise from the beginning in order to better understand the problem being addressed.

Some participants suggested that synthetic biology might be making exaggerated claims about solving global challenges and cautioned that this could lead to undesirable effects: (1) disenchantment if promises are not met; (2) neglecting alternative approaches; (3) shaping the next generation of experts.

Several participants suggested that the current focus on demonstration of impact in research grants can lead to applicants making exaggerated claims about the speed and scope of the future economic and social impact of their work. One participant also noted that the iGEM competition[3] pushes students to think forward in a futuristic way, which leads to exciting and creative proposals, but could also result in a naive attitude towards the problem teams were trying to solve. Caution was urged about over-promising on the applications that synthetic biology can deliver, particularly in terms of environmental applications, as this could be damaging to the field in the long-term if the promises are not met.

Some participants (especially those not engaged in synthetic biology research) also mentioned their perception that research councils in the UK would be more likely to fund projects that mentioned synthetic biology, and that this encourages existing areas of research to try to rebrand themselves as synthetic biology. They felt that this has an undesirable effect because it can lead to other interesting approaches being neglected.

[3] The International Genetically Engineered Machine (iGEM) competition is a competition for undergraduate students that has played a key role in the global development of the field of synthetic biology. Based on a kit of biological parts, teams work over the summer to design and build biological systems and operate them in living cells. See: www.igem.org

A participant gave an example of one such alternative approach: instead of trying to develop agricultural applications based on the release of a GMMO, it is now possible to use recent advances in molecular biology to better manage natural processes. For example, next generation sequencing and improvements in PCR techniques can be used to monitor how different agricultural management strategies affect whole microbial communities and the expression of genes of interest, such as those involved in the nitrogen cycle. It is also possible to go on "fishing expeditions" to collect naturally occurring microorganisms that may be able to perform particular functions. But this participant felt that such approaches would be less likely to be supported by public research funding bodies than synthetic biology approaches.

Some participants felt that because synthetic biology is seen as a new and promising field, it is attracting young and smart researchers and that this means that fewer would want to study other fields such as microbiology or ecology, which are fundamentally important to understanding systems as a whole. These participants also expressed the view there was a difference between what they called "classical microbiology" training from decades ago and the new kind of education provided to molecular biology and synthetic biology students today. They felt that "classical microbiology" training was broader and provided a better understanding of living organisms and whole systems, which helped to produce people who could understand and adapt to new questions and problems throughout their working lives[4].

[4] Following the workshop, some synthetic biologists have argued that, compared to other disciplines, synthetic biology students receive a very broad range of training from multiple disciplines including engineering, mathematics, microbiology, molecular biology, design, social science, ethics; and that the 'human practices' element of the iGEM competition provides an unusual and valuable extra dimension to synthetic biology education.

SESSION 2: Regulation for contained use and deliberate release of GMMOs

This session explored how regulations in the USA, the EU and the UK distinguish between and deal with deliberate release and contained use of GMMOs. It also aimed to examine the way in which social and institutional factors challenge the regulatory distinction between deliberate release and contained use[5].

Presentation by Dr Mark Segal
United States Environmental Protection Agency
"Synthetic Biology and the Coordinated Framework"

The Coordinated Framework for the Regulation of Biotechnology (1986) provides an umbrella for US regulations on biotechnology, with overlap of responsibilities among the agencies (FDA, EPA, USDA, OSHA, NIH). Genetically engineered micro-organisms for commercial products are regulated by the EPA Toxic Substances Control Act (TSCA), which was conceived for toxic chemicals and now also includes microorganisms. Through the Coordinated Framework policy statement and the TCSA Biotechnology Rule (1997), "new" micro-organisms were included as substances within the authority of TSCA. "New" or "intergeneric" micro-organisms are defined as those formed by deliberate combinations of genetic material that comes from organisms from different taxonomic genera; or constructed with synthetic genes that are not identical to DNA that would be derived from the same genus as the recipient microorganism. Naturally occurring micro-organisms, genetically engineered microorganisms other than intergeneric, and intergeneric organisms resulting only from the addition of well-characterised, non-coding regulatory regions are excluded from new microorganism review. Furthermore a number of application areas are excluded from the TSCA including pesticides, food, drugs, and cosmetics.

Any manufacturer or importer of a new micro-organism must file a Microbial Commercial Activity Notice (MCAN) 90 days prior to initiating manufacture/import. Any person wishing to introduce a new micro-organism into the environment for commercial R&D purposes must submit a TSCA Experimental Release Application (TERA) 60 days prior to initiation of the field test. A risk assessment is conducted for human health and ecological impacts, as well as an economic analysis. The TSCA is a Risk/Benefit statute, which means that both risks and benefits are taken into account in the regulatory decision. This can take three routes: sufficient information to determine "no unreasonable risk"; sufficient information to determine "unreasonable risk"; or insufficient information to determine effects.

A person who manufacturers, imports or processes a GMMO is not subject to MCAN reporting requirements if the activities are conducted inside a structure, defined as "a building or vessel which effectively surrounds and encloses the microorganism and includes features designed to restrict the microorganism from leaving." The definition of "vessel" was left intentionally vague. The EPA has to anticipate all the possible uses of the new micro-organism, not only the uses that are described in the notification, because once approval has been granted, the organism can be used for any purpose, and/or by people other than the applicants. Research conducted in non-commercial settings generally does not fall under EPA oversight.

[5] For further detail of these regulations and these issues, see the Scoping Report for this workshop.

Presentation by Dr Martin Cannell
GM Team, UK Government Department for environment, food and rural affairs (Defra)
"UK/EU regulations"

Contained use of GMOs is regulated by EU Directive 2009/41/EC, and deliberate release is regulated by EU Directive 2001/18/EC, which were transcribed into UK Regulations in 2000 and 2002 respectively. There is extensive guidance for implementation of these regulations including "A guide to GMOs (Contained Use) Regulation 2000" and the "SACGM[6] Compendium of Guidance on genetic modification". The principles underlying the regulation of deliberate release are: that each GM organism release is considered on a case-by-case basis; that the GMO is to be released in a stepwise fashion whereby the scale of the release increases and risk management decreases as knowledge about the GMO and its interaction with the environment increases; that decisions are based on an assessment of risk and associated risk management procedures; and that authorisation for placing on the market can only occur after adequate field testing in relevant ecosystems. The current regulatory appraisal system only considers the potential risks of a product and does not take into account potential benefits.

Defra considers that the regulatory system is generally fit for purpose and able to capture the majority of synthetic biology applications, but blurred boundaries between contained use and deliberate release will likely need to be addressed as applications move beyond R&D and into the market. The Health and Safety Executive (HSE) and Defra work closely together to address these blurred boundaries and applicants are encouraged to contact HSE and Defra to discuss these on a case-by-case basis. A recent gene therapy clinical trial was a case in which the decision about whether to regulate as contained use or deliberate release was unclear, because some shedding of the introduced genetic material from the patient was expected. The applicant contacted both the HSE and Defra and a pragmatic regulatory approach was devised collaboratively. Ultimately the deliberate release route was chosen. The environmental risk assessment was discussed by the Advisory Committee on Deliberate Release (ACRE), who advised that consent should be given because of the high level of biological containment. In light of the experience from this case, policy is being reviewed to consider whether clinical trials in which GMOs have a very high level of biological containment could be appropriately dealt with under the contained use regulations, irrespective of the degree of shedding.

The case of the Wellcome Trust-funded arsenic biosensor project described in the workshop Scoping Report is also interesting in this respect. The researchers involved are attempting, with advice from Defra, to use a clause in Directive 2009/41 that raises the possibility of establishing a list of GMMOs that are considered safe enough to be exempt from the scope of the Directive[7].

[6] Scientific Advisory Committee on Genetic Modification.

[7] See workshop Scoping Report and http://www.arsenicbiosensor.org for details.

Presentation by Prof. Hauke Harms
Helmholtz Centre for Environmental Research
"The ARSOlux experience"[8]

Researchers at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig, Germany have developed a bacterial biosensor (*Escherichia coli*) to detect arsenic in ground water. The ARSOlux team patented the technology in 2003 and the first tests were conducted in Vietnam in 2005. In 2010 field tests were conducted in Bangladeshi villages. The product tested consisted of living GM bacteria contained in a sealed vial. The tests demonstrated good reliability as well as increased simplicity of use and reduced waste compared to competitor products using chemicals. However, while arsenic water contamination is a major health problem in Bangladesh, and despite having a reliable product and spending time speaking with government representatives in Germany and Bangladesh, the team have been unable to secure approval from the Bangladeshi government for commercial use of the device. The political instability in the country presented major challenges and the team are now exploring other potential markets.

During their trips to Bangladesh, the team were frequently asked whether they had received regulatory approval to use the product in Germany. The team had not envisaged using their biosensor in Germany because arsenic water contamination is not a serious problem there, but in order to demonstrate their willingness to comply with regulatory authorities, the team sought approval for a field experiment in Saxony. This was granted under 'contained use' regulations, and under the condition that the tests be conducted in a 'lab in a van' by trained scientists.

Key lessons learned from the ARSOlux experience were that a GMMO product needs to demonstrate its unique or superior selling point, and that getting a product through to commercialisation takes time, money and stamina.

The team is now working on a different product design, similar to a pregnancy test strip that changes colour when dipped in liquid. In this case, the aim would be for the product to be used by untrained people. With this product there is a greater probability of dispersal of the GMMOs because it would not have the same level of physical containment as the current ARSOlux product (as it would not be in a vial).

DISCUSSION

Participants discussed the relevance of physical containment for risk assessment of GMMOs.

It was generally agreed that physical containment in a vial, as used for the ARSOlux product, can never be fail-safe: the vial might break, or local people may wish to reuse the vial, for their own purposes even if this goes against the instructions. For some participants this meant that the question 'what might happen if and when it gets out?' should be part of risk assessment conducted as part of the regulatory process. Others, who considered that the organism used by ARSOlux was inherently safe (since it uses a bacterial strain recognised by the German authorities as non pathogenic and able to survive only a limited time outside the laboratory), thought that a request for further evidence of safety would be excessive; and that some low level of relative risk should be considered acceptable. In response, one participant wanted to know what tests had been conducted to demonstrate the safety of the strain, and whether the data had been made publicly available for peer review. For this participant considering what might happen if a GMMO escaped from physical containment was particularly important because of the ability of living organisms to replicate and/or pass on the altered genetic information.

One participant introduced the idea of 'geographical containment', suggesting that release of GMMOs should be prevented in particularly sensitive or valuable geographical areas.

Participants discussed the importance of the context of use in determining whether a device constituted 'contained use' or 'deliberate release'

Several participants remarked that the level of training of the user and the social and institutional conditions in which the measurement was conducted when the ARSOlux biosensor was field tested in Germany was very different to the envisaged goal of providing the device to villagers in Bangladesh. Although the German field trials conducted by scientists in a lab-in-a-van were defined as 'contained use'

[8] Slides for this presentation are not available. For further information see: http://www.ufz.de/arsolux and the Scoping Report for this workshop.

this did not necessarily mean that the use of the same device by different users and in a different social context would or should also be considered as 'contained use' by regulatory authorities.

The importance of assessing benefits as well as risks was stressed by many participants, for various reasons: (1) it could help speed up regulatory decisions for applications with seemingly obvious humanitarian benefits; (2) it could help challenge unwarranted claims about benefits; and (3) it could help open up deliberations about the nature of benefits.

The idea that the US regulatory procedure includes an economic analysis and takes into account benefits as well as risks, whereas the EU/UK procedure currently only considers potential risks, was discussed at some length. Many participants argued that there should be more of an emphasis on benefits in the EU regulatory process, although there were different perspectives about what this might achieve. Some participants suggested this would help to get synthetic biology applications through the regulatory system where they appear to have obvious humanitarian benefits. Others argued that a methodological assessment could challenge unwarranted claims about the problem at stake, as well as raise questions such as: who benefits, and under what circumstances? What alternative approaches exist or could be developed to deliver these benefits? How can we conduct a fair comparison between different options?

One participant remarked that socio-economic assessment of benefits (and risks) has been discussed for some time at the European Commission, where it is sometimes referred to as 'the fourth hurdle', and was nearly included in the EC Directive for deliberate release in 1990[9]. Such assessments would require novel types of trans-disciplinary research. Referring back to discussions earlier in the day about 'technologies in search of a problem' and the way in which research funding decisions influence which approaches are likely to be developed, this participant argued that socio-economic assessment of benefits could perhaps help redirect research towards technologies that would work in realistic conditions, and to identify the conditions necessary to reliably deliver benefits.

[9] A European Socio-Economic Bureau for GMOs (ESEB) was recently established by the European Commission. For further details see "Summary report for the Workshop 'Framing socio-economic assessment in GMO & chemicals regulation', European Environment Agency, Copenhagen, 6-7 December 2012" available at: http://www.umweltbundesamt.at/umweltsituation/gentechnik/gentechnik_termine/socio-economics-gmoschemicals/

SESSION 3: Horizontal gene transfer and engineered biocontainment

This session examined the engineering approaches to biocontainment that have been proposed since the 1970s by microbiologists and synthetic biologists and explored the extent to which they provide solutions to biosafety concerns.

Presentation by Dr Ollie Wright
CSynBI, Imperial College London
"Built-in biosafety design" [10]

Two key biosafety concerns relating to the release of GMMOs into the environment were identified at the 1975 Asilomar Conference: GMMOs might outcompete indigenous organisms; and genetic contamination might occur through horizontal gene transfer (HGT). These biosafety issues are actively discussed within the synthetic biology community, and a number of built-in biocontainment measures have been proposed to prevent HGT and dispersal. Several iGEM teams have focused on this issue, for example the Paris Bettencourt 2012 team bWARE project that proposed a triple biosafety system including different 'kill switches'. A weakness of such systems is that organisms need only a few mutations to inactivate a toxicity-causing device. A gain-of-function is much harder to achieve evolutionarily than a loss-of-function, and this should be taken into account in biosafety designs.

Transformation (sequence-independent uptake of free DNA from the environment) is harder to mitigate than conjugation (active transfer via pili) or transduction (active transfer via bacteriophages) mechanisms for HGT. Minimising genes with an obvious selection advantage and including genes with an obvious selection disadvantage could improve biosafety. Flanking sequences should be as different as possible form naturally occurring sequences to minimise the chance of homologous recombination into other genomes. It would also be best to use microbes that do not survive long when released into the environment (e.g. with multiple attenuations) rather than relying on kill switches. Semantic containment (e.g. shuffling of the genetic code, xeno-nucleic acids) was also mentioned as a future biosafety mechanism.

Monitoring rates of HGT in bacterial populations is challenging due to the large sample sizes or long timeframes required when looking for these rare events; if the selection coefficient is weak, the time required to infer the occurrence of a HGT event may be 10 to 1,000+ years. As a test-bed for the efficacy of biosafety designs, a strong selection coefficient for HGT should be incorporated into a worst-case-scenario laboratory trial.

In conclusion it was suggested that it is important to incorporate multiple biosafety mechanisms in order to ensure system redundancy, whilst minimising the fitness cost to the GMMO product; and that systems based on full deletions and complementations were preferable to those based on more minor inactivation of a gene.

[10] Further details are available in Wright, O., Stan, G.-B., & Ellis, T. (2013). "Building-in Biosafety for Synthetic Biology." *Microbiology*, 159: 1221-1235.

Presentation by Prof. Pascal Simonet,
Environmental Microbial Genomics Group, Ecole Centrale de Lyon
"Horizontal gene transfer in the environment"

The important question for applications involving environmental release of GMMOs is how to make organisms that will actually survive and persist effectively in the environment in order to perform their intended function. Natural ecosystems are complex, with many different microbes interacting. Almost all the attention in risk research, risk assessment and policy discussions has been and still is about how to make sure the genetically engineered organisms do not survive, persist, and 'take over' natural ecosystems: 'the superbug question'. However, this is the wrong question. The right question is: how can we make efficient and competitive micro-organisms that can perform their intended job in a hostile natural environment? Currently 'natural' microbes are better at doing that than GM microbes.

Horizontal gene transfer (HGT) is a necessary and critical mechanism for adaptation and evolution. One example to demonstrate the importance and versatility of HGT comes from bacteria that are able to produce a great variety of polyketide antibiotics by shuffling and transferring gene domains among bacterial populations. The functional diversity of the variants of polyketides produced by natural bacteria is much greater than for chemically synthesised molecules generated by random chemistry.

A second example is the detection of a bacterium (*Sphingobium francense*) that is able to degrade lindane, a toxic pesticide, in highly polluted soils. Lindane was first synthesised in 1946 and no similar compound is found in nature, so these bacteria must have evolved a new metabolic pathway to degrade it. Such degradative bacteria cannot be detected in non-polluted soil. The degradative bacteria contain a *linA* gene that is involved in the first and essential step in the metabolic pathway. Research has demonstrated that this is not an ancestral gene that has been reactivated, but rather an entirely new mosaic gene, created by assembling four DNA fragments acquired via HGT from other bacteria. This process required numerous transfer events over a short period of time (a few years) and demonstrates how HGT is involved in rapid (but not optimised) responses to new environmental conditions. This illustrates two possible targets for synthetic biology: *de novo* generation of new functions, or optimisation (fine tuning) of the products of natural evolution. It would have been very difficult for humans to design a new metabolic pathway and the associated *linA* gene, but synthetic biology tools could be used to optimise it.

There are three types of barriers to HGT *in situ*: physical (contact between recipient and donor organisms, or their DNA), physiological (active metabolism of donor and recipient bacteria), and genetic. A crucial criterion for HGT is the presence of homologous DNA sequences that create 'hot spots' for gene transfer. When the first risk assessments for GM crops were conducted in the 1990s, it was assumed that there could be no transfer of genetic material between a GM plant and soil bacteria, but this has now been shown to occur, through homologous recombination.

HGT, especially among bacterial populations, is unavoidable. HGT is the main adaptive weapon of the best adaptable organisms on this planet. Engineered safety mechanisms based on genetic devices can reduce the probability of HGT but cannot eliminate it. Multiple devices may increase biosafety efficiency but will never offer a total guarantee. Moreover, as pointed out by Wright et al., the higher the complexity of the safety device, the higher the physiological burden on the host and in turn the higher selective pressure to circumvent the biosafety mechanism.

The fact that HGT is unavoidable does not, however, mean that negative impacts on the environment will be high. The impact is likely to be negligible because wild bacteria are too strong. The high taxonomic and functional microbial diversity in the environment, the heterogeneity and complexity of natural media, and the harsh selective pressure that bacteria and other micro-organisms face make it difficult for man-made micro-organisms to outcompete native species and disrupt habitats.

DISCUSSION

There was general agreement that engineered biocontainment based on built-in genetic designs may be able to decrease, but could not completely eliminate, the frequency of Horizontal Gene Transfer (HGT) and the likelihood of persistence and dispersal of released micro-organisms. Participants had different opinions about the implications this has for the role of engineered biocontainment for biosafety.

There was general agreement that built-in genetic designs for biocontainment could never be fail-safe: they may be able to decrease, but could not completely eliminate, the frequency of Horizontal Gene Transfer (HGT) and the likelihood of persistence and dispersal of released micro-organisms. Bacterial populations have well-known mechanisms, including HGT, that can enable them to evolve and circumvent biosafety designs. Therefore, given high enough population numbers and/or long enough timescales, bacteria may override any kind of 'kill switch'. Participants had different opinions about the implications this has for the role of engineered biocontainment for biosafety. For some participants, the focus should be on trying to improve genetic biocontainment designs and to better quantify the incidence of HGT using mathematical models and/or field experiments. Others suggested the focus should shift towards research to evaluate the potential consequences on human health and the environment *if* HGT occurred and/or the GMMO did persist or disperse following the deliberate release of a specific GMMO into the environment. Some synthetic biologists thought that biocontainment could, in circumstances where the potential negative environmental impact of HGT or dispersal was assessed as low, be considered an appropriate approach to help maximise biosafety. Other participants (including NGOs, microbiologists and regulators) stressed that engineered biocontainment is not by itself an adequate biosafety approach. Regulators present emphasised that the key question for risk assessment was to assess whether or not the GMMO released could be associated with potential harm, , and that HGT in itself does not constitute a hazard[11].

The discussion, in this session and throughout the day, illustrated that there is a balance to be reached between making GMMOs survive long enough in the environment to perform their intended function, and aiming to contain the released organisms and the inserted or modified genetic material in order to reduce the potential for environmental harm.

There was a general agreement that most of the attention in risk research, risk assessment and policy discussions surrounding environmental risks has focused on how to make sure that the genetically engineered organisms released into the environment (or other organisms that pick up their genetic material) do not survive and 'take over' natural ecosystems, i.e. 'the superbug question'. Some participants suggested that this might be the wrong question and that the real challenge is how to create micro-organisms that could compete well enough with pre-existing bacterial populations in order to perform reliably and effectively in the environment. This was essential for the GMMOs to deliver their intended benefits and/or to be of commercial interest.

A number of participants argued that there was a case for developing GMMO applications that would specifically *not* need to be able to compete effectively with wild bacterial populations, either because they were physically contained (e.g. in bioreactors or glass vials), or because they would only be required to survive for short periods in the environment. For some participants, the key advantage of such containment or 'semi-containment' is that the environment can be controlled to some extent, and that this would help ensure the viability of GMMOs as useful products. Others focused on semi-containment as a means to help ensure safety.

Participants discussed the difference between product-based and process-based regulations and their implications for how GMOs are treated differently by US and EU/UK regulatory systems.

The example of the *linA* gene demonstrated how natural selection can lead to elegant solutions to problems and led to discussions about how synthetic biologists could perhaps accelerate such processes in the lab, or in field environments especially designed to increase selection pressure and encourage HGT. This raised the question of whether, in such cases, the resulting organisms would be classified as GMOs according to existing regulations. Regulators responded by saying that boundaries between GM and 'naturally' selected organisms have been discussed at length since the 1990s, especially in the context of

[11] In UK and EU regulations HGT is not defined as a hazard in itself but rather as a mechanism through which adverse effects could occur. See Scoping Report for further detail and discussion.

plants[12]. In the US, it is mostly the end use of the product that determines whether and by which agency a GMO is regulated; and if the genes inserted are present in other organisms from the same genera then the engineered organism does not fall within the scope of EPA Toxic Substances Control Act (TSCA). In the EU, it is mostly the process used to insert the genes which determines whether an organism falls under GMO regulations. Thus, an organism defined as a "GMMO" within UK/EU regulations will not necessarily be defined as an "intergeneric micro-organism" within US regulations[13].

For some participants, these regulatory definitions appeared to be somewhat illogical, and they could think of examples of organisms that could be produced by laboratory researchers that would fall outside the scope of US and/or EU regulations, and yet would not necessarily be inherently less hazardous. A regulator remarked that some companies were indeed thought to be seeking to exploit such gaps to find ways to escape regulatory oversight, but that this was not advisable. Another participant suggested that even if regulations have slightly illogical 'triggers' to bring things into the review system, this is not necessarily a significant problem: sometimes regulations will catch things that are not problematic and those cases can get through the system quite swiftly, and things that are not captured by GM regulations can perhaps be effectively captured by other regulations to assess their safety.

Participants discussed the relative weight that should be given to knowledge about DNA sequences when assessing environmental risk

One participant expressed concern that risk assessments were too narrowly based on knowledge of linear DNA sequences, which is not sufficient to judge harmfulness. They noted that the same genes placed in different organisms or different contexts have been known to function differently, so it is not possible to reliably predict how a known gene sequence will behave in a new genetic and cellular environment. On the other hand, another participant argued that knowledge about the DNA sequence inserted into the engineered organism was the most crucial piece of information, and furthermore that if such a sequence is already present in the environment then harm is unlikely to occur.

One participant stressed the need to take into account radical uncertainty

A social scientist pointed out that we are in a situation in which we need to act in the face of "radical uncertainty"[14]. There are examples of innovations that were assumed to be safe and then were shown to cause serious environmental harm, and we can learn lessons from these cases. This participant insisted that this is not about being 'for' or 'against' synthetic biology, and is not about stifling innovation. Innovation systems are complex, just like soil microbiology, and a rigorous balanced approach to dealing with knowledge in conditions of uncertainty is needed. It is important to take care not to speak in particular ways that have become routine and which have caused problems in the past, in particular: (1) assume that when something is 'natural', it is necessarily safe; (2) assume that if something is 'low risk', we can go ahead; (3) assume that containment is always possible and is an appropriate solution to uncertainty.

[12] The question then was whether and how to distinguish between conventional plant breeding and GM techniques to produce crops.

[13] See workshop Scoping Report for further details.

[14] The term "radical uncertainty" was used here to refer to the concept first proposed by Frank Knight in 1921 (in his book *Risk, Uncertainty, and Profit*) and further elaborated since then by other scholars in economics and social studies of science and technology (Keynes, Shackle, Collingridge, Smithson, Ravetz, Wynne, Stirling). For a discussion, see Stirling, A. (2010). "Keep it Complex." *Nature*, 468: 1029-1031.

SESSION 4: Broader perspectives on risk and uncertainty

This session examined current governance frameworks dealing with risk and uncertainty and how any limitations could be addressed.

Presentation by Dr Ricarda Steinbrecher
Econexus
"Perspectives on risk and uncertainty"

The talk began with a list of historical examples, demonstrating that risk assessments and decisions for GMOs have at times been based on unproven assumptions, false predictions and lack of knowledge. For example 'naked' DNA was initially assumed not to persist in the environment, but it has now been shown that it can survive for long periods in certain situations. rDNA techniques for GM crops were assumed to be precise, and when combined with the knowledge of the DNA sequence inserted, this was assumed to lead to increased safety, but this ignored transformation-induced mutation effects across the plant genome and at the insertion site, that resulted in imprecision and unpredictability[15]. Another example was the Cauliflower Mosaic Virus (CaMV) 35S promoter, widely used for GM crops. It was initially assumed that this promoter, because of its plant virus origin, was not functional in bacteria and thus that even if HGT occurred from GM crops to bacteria, genes that were driven by that promoter would not be expressed. Statements asserting this continued to be made by EU and UK advisory bodies and be used in risk assessments submitted to regulatory authorities for GM plants, even after a number of articles were published demonstrating CaMV 35S promoter activity in bacteria, yeast and human cells (see examples in slides)[16]. More recently it has also been demonstrated that the 35S promoter unexpectedly contains an open reading frame. Paying attention to these examples is not about being 'pro' or 'anti' GMOs, but about doing good science and checking facts. It is also about addressing uncertainty as a crucial and scientific component in risk assessment.

The 'Push-Pull'[17] technology widely used in East Africa to tackle stem borer infestations and the parasitic Striga weed is a good example of a locally researched system-based approach. In contrast to approaches based on using an isolated compound such as strigolactone (which may lead to altered responses and undesirable effects on mycorrhiza), it takes into account the whole complex agro-ecosystem, provides a range of side benefits (e.g. the provision of all-round quality fodder for livestock, nitrogen fixation and reduced soil erosion) and provides a more sustainable solution. Similarly the use of artemisinin as an isolated compound to treat malaria is likely to lead to resistance problems, whereas remedies based on whole *Artemisia* plants can be more effective and are reported to be less likely to lead to resistance.

Biological containment should not be thought of as contained use, because biological measures can be overcome by the organisms and are therefore not reliable. Biocontainment strategies developed for plants (Genetic Use Restriction Technologies, GURTs), insects (Release of Insects containing a Dominant Lethal, RIDL) and fish (triploidy) have claimed sterility but in practice they have not achieved it reliably.

There is a tendency for synthetic biology to over-simplify, to treat living systems as if dealing with mechanical systems, to focus on technology as the only solution to a problem, and to ignore locally-researched solutions, root causes and system solutions. There is also a tendency to think that the availability of technological answers means that we do not need to change our human behaviours that are causing the problem, for example focusing on bioremediation rather than reducing pollution. 'Techno solutions' that focus on symptoms ignore surrounding systems and as a result are often unreliable and short-lived, and lead to another layer of problems that can be even harder to deal with. 'System solutions' are more durable and sustainable in the long term, but require a very different approach.

[15] For further details see: Wilson AK, Latham JR and Steinbrecher RA (2006). "Transformation-induced Mutations in Transgenic Plants - Analysis and Biosafety Implications." *Biotechnology and Genetic Engineering Reviews*, 23: 209-237

[16] For further details see: Steinbrecher RA (Dec. 2002). The CaMV 35S promoter. EcoNexus Briefing. http://www.econexus.info/sites/econexus/files/ENx-CaMV-35S-Promoter-B-2002.pdf

[17] See workshop Scoping Report for the description of a synthetic biology based approach to tackling Striga, and this website for more information about the Push-Pull technology: http://www.push-pull.net/striga.shtml

Presentation by Prof. Andy Stirling
SPRU, Sussex University[18]
"Risk, uncertainty… and the governance of technology"

The presenter began by stressing that he is not being specifically critical of synthetic biology, but wanted to talk about some general implications of power, which, though very real, are often not spoken about because they are thought to be impolite. For instance, government strategies proclaim 'pro-innovation' policies as if innovation moves forward on a single linear track in any particular technological field, with no effective alternative pathways. Being critical of any particular path for science and technology, such as synthetic biology, is treated as if counter to progress itself. But the choice is not about being 'for' or 'against' innovation. Innovation does not just have speed, it also has direction: there are choices to be made between different options. Key questions are: which way should we go? Says who? Why?

Science and technology takes place in political, economic and social systems. Innovation is an irreversibly branching evolutionary process, and the regulatory system we have been discussing is part of this innovation system. As a result, not all that is scientifically realistic, technically practicable, economical feasible or socially viable will be historically realisable. As certain options are closed down, certain technology pathways get 'locked-in' and choice can become artificially closed.

Risk is a narrow concept, and is radically different from uncertainty. Risk approaches are appropriate only when knowledge about likelihoods is unproblematic (e.g. for high frequency incidents in familiar contexts). Such cases are, however, rare in practice and it is necessary to move beyond a narrow focus on risk. Current regulatory practice tends to focus on risk and as such fails to address situations of ambiguity, uncertainty and ignorance. In situations of uncertainty, knowledge about likelihoods is problematic (e.g. low frequency events or where human factors and changing contexts play an important role). In situations of ambiguity, knowledge about likelihoods may be unproblematic but disagreements exist about what matters, and what counts as 'harm' and 'benefit'. This leads to a different set of questions. Rather than taking one product and asking 'is this safe?', or 'is this at least no worse that the least safe existing comparator?', we might ask 'which alternatives would be best?' and 'what kind of society do we want to live in?'. Ignorance occurs in situations where knowledge about both likelihoods and possibilities is problematic. Here we have surprises, and sometimes also wilful blinkers.

Science-based regulatory systems, political culture, liability, insurance, and over-emphasis on metrics and quantitative methods all tend to push practices and policies away from dealing with ignorance, ambiguity and uncertainty, and towards treating problems only in terms of risk. We are in what sociologist Ulrich Beck has called a state of "organised irresponsibility".

A narrow focus on risk leads to attention being placed mostly on measuring and modelling probabilities of adverse events and ensuring that identified risks are 'low', with a focus on prediction ('more numbers') and (bio)containment. Complexity and uncertainty should be acknowledged by scientists, and within the regulatory process. Paying more attention to irreducible uncertainties leads to more attention on acknowledging unpredictability, un-containability, irretrievability and irreversibility, which in turn leads to seeking new procedures for decision making that are more transparent, plural and democratic.

A narrow risk approach demands definitive prescription, whereas a reflexive, precautionary and participatory approach to governance opens up more options, issues, uncertainties and perspectives, and seeks to reconcile scientific rigour and democratic accountability. Experts are often expected to give definitive answers, but a scientifically rigorous answer will often be 'it depends on how you look at the question'. This also means that a shift is needed from risk governance to innovation governance. And we should not feel so anxious about terminating one avenue when there are better alternatives.

[18] For further details see Stirling, A. (2010). "Keep it Complex." *Nature*, 468: 1029-1031.

DISCUSSION

One synthetic biologist was unhappy that synthetic biologists seem to be cast as seeking to "technologize" solutions to all of the world's problems.

This participant acknowledged that solutions based on isolated compounds may perhaps be problematic in some cases (e.g. Striga), but not in others. They also enquired whether there was evidence to demonstrate that the semi-synthetic artemisinin was more likely to lead to resistance than other malaria treatments[19]. They argued that they, as a synthetic biologist, were interested in comparing their envisaged synthetic-biology-based solution with alternative approaches.

The discussion returned to earlier themes about over-promising and the role of funding bodies in shaping research priorities. Some participants suggested that funding mechanisms needed to be developed for technology assessment and trans-disciplinary research.

The tendency to make bold promises in grant application was again brought up. However, it was pointed out by one participant that funding applications do go through peer review processes and reviewers do question overly-fanciful claims, which can lead to those applications being declined.

It was noted by one participant that data on how much public money in the UK is devoted to different science and technology streams is not available, despite this evidence being requested in a House of Lords Select Committee Report in 2010[20]; and this data would be essential for better and more transparent deliberations about research priorities. In addition, 25-50% (depending on the definition used and sources of information) of global research funding comes from military institutions and even less data is publicly available about this.

Participants suggested that funding mechanisms needed to be developed for technology assessment (TA) and trans-disciplinary research to identify the challenges that need to be addressed and open up comparative analysis of different approaches, rather than taking one single technology and seeking the best way to develop it to market. One participant suggested that a proportion of research funding should be set aside for TA, even if this meant less funding for scientific research. They remarked that it is accepted that scientific research to produce innovation will take years, but rigorous evidence to inform policy is expected to be generated instantly. Another participant cautioned that it would be important to ensure that the TA methods used did not close down options.

In a recent Technology Strategy Board (TSB) funded feasibility studies competition, £0.5 million had been set aside by the Economic and Social Research Council (ESRC) for social science research, but none of the applications received contained relevant components so the money was not allocated. Some participants suggested that this could be due to the requirement that projects be business led. One social scientist argued that the remit of that specific TSB call was too restrictive, in that it asked only 'how should we implement particular applications of synthetic biology?' rather than encouraging questions about why synthetic biology is the most appropriate approach and what alternatives may be available. Another social scientist argued that we undermine science when we fail to recognise its versatility and that research needs to ask 'what are the right challenges?'. In order to do this, we would need genuinely trans-disciplinary kinds of research (not just social science research).

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[19] In her talk, Ricarda Steinbrecher made a contrast between malaria treatments based on whole Artemisia plants and treatments based on the isolated artemisinin compound, regardless of whether the artemisinin drug was produced via synthetic biology, and regardless of whether or not it was administered as a combination therapy with a second isolated pharmaceutical compound. Subsequent discussion revealed that this point was largely misheard, with synthetic biologists believing that the critique was targeted at semi-synthetic artemisinin produced via synthetic biology as opposed to the same compound extracted from the plant.

[20] House of Lords Science and Technology Committee (2010). Setting Priorities for publicly funded research. 3rd Report of Session 2009-10. Stationary Office: London.
http://www.publications.parliament.uk/pa/ld200910/ldselect/ldsctech/104/104i.pdf

SESSION 5: General discussion

The session began by asking the synthetic biologists present to provide their comments on the discussion so far. Some of the points mentioned by one or several synthetic biologists were:

- They had found discussions about the regulatory definitions of 'GMOs' and 'intergeneric organisms' informative.
- The historical examples provided useful context.
- They recognised the need to acknowledge uncertainty and to consider the views of different stakeholders.
- They appreciated that turning proposed environmental applications into real world products is a challenge, and that the proposed benefits, as well as the wider environment, need to be taken into consideration alongside technical feasibility.

Discussion of 'broader questions' and responsibility

Almost all the synthetic biologists used this opportunity to return to themes relating to what they referred to as 'broader issues', 'big questions' and 'social impacts', and to the critique that synthetic biology was a 'technology looking for a problem'. This led to a wide-ranging discussion about responsibility**.**

The synthetic biologists generally agreed that there were real and important 'broader questions' about the purpose of their work, but argued that this was not unique to synthetic biology, and queried whether they, as research scientists working at the bench, should be expected to address these broader questions. For example, putting *Artemisia* farmers out of business, or considering which forms of artemisinin treatment is more likely to lead to resistance are real issues, but a number of synthetic biologists present thought that these considerations were beyond the remit of their jobs and/or beyond their realm of expertise. Another synthetic biologist took a slightly different position and argued that these broader issues cannot be ignored if research is being taken beyond the bench to product development. This participant suggested that this has to be done by working with people with other kinds of relevant knowledge (e.g. microbial ecologists).

The allegation that synthetic biology is a 'technology looking for a problem' was contested by one synthetic biologist who viewed this as an unwarranted criticism of the field. He noted that there were historical examples of technologies (e.g. lasers) that were developed by scientific researchers who did not and could not have anticipated the wide-ranging applications that resulted in the long term.

These opening remarks from synthetic biologists led to a discussion with the whole group about whether it is fair or appropriate to expect synthetic biologists to address all the social implications of their work, and whether and how others (social scientists and/or stakeholders) should be involved. Some synthetic biologists felt that they were being asked to be personally responsible for all the implications of their work, but social scientists and NGO representatives clarified that they did not wish synthetic biologists to be solely responsible for all impacts of their research when working at the bench and focused instead on the responsibility of synthetic biologists who are promoting the field to temper their claims and to acknowledge uncertainty. One social scientist pointed out, however, that some scientists also play important and privileged roles outside of their laboratories (e.g. when participating in public debates or sitting on regulatory and advisory committees), and suggested that in those cases they should assume more responsibility.

A social scientist pointed out that when scientists and others speak (outside of the laboratory setting) to promote a particular field, conditionals tend to disappear. So "synthetic biology *could*…" quickly changes to "synthetic biology *can*…" and then to "synthetic biology *will*…". This way of speaking is part of the problem, as it makes it more difficult to ask questions about: benefits to whom? Under what conditions? And with what downsides?

A social scientist acknowledged that social science also has its own hype, but that this is perhaps not so problematic because social scientists appropriate far less resources and their claims are seldom taken as seriously.

One participant pointed out that the current UK government had identified synthetic biology as a key area for public investment, with many millions of pounds now committed to the field. This is a great opportunity for synthetic biology, but could also be seen as detrimental to the field, because it leads to greater pressure to deliver successful commercial applications in the short-term.

Uncertainty

The final part of the discussion returned to the issue of uncertainty. One participant argued that regulatory and policy discussion did not focus enough on uncertainty. For example, we should accept that containment is leaky, and be prepared for surprises. In response, a synthetic biologist asked whether this meant that there should be more research to determine the probability of HGT. A social scientist argued that uncertainty tends to get dropped when science is used for policy, and that advisory science is not good at addressing 'unknown unknowns'.

CONCLUDING REMARKS

This workshop did not seek to arrive at consensual views or to generate specific recommendations, but the executive summary draws together the main themes that emerged during the day. A key aim of this workshop was to promote constructive discussion and better mutual understanding on the prospect for synthetic biology that would require release into the environment, between actors with very different perspectives. Feedback received from participants (see below) suggests that this aim was achieved.

Feedback received

A feedback questionnaire was distributed and very positive feedback was received from workshop participants, with many comments focusing on the diverse mix of speakers and participants, and the value of bringing together people with different backgrounds and perspectives. Comments also focused on the clear aims and structure of the workshop agenda, which enabled interesting, open and constructive discussion. However, it was also suggested that the workshop could have been longer in order to take the discussion further.

Some of the key themes identified in the feedback received were: the complexities involved in regulatory definitions of contained use and deliberate release; the need to acknowledge the complexity of the environment; the importance of acknowledging uncertainty; and the role of wider stakeholder participation. Two thirds of respondents agreed or strongly agreed that their participation in the workshop will have an impact on their future work.

Responses to the question "What was best aspect of the workshop?"

"Good mix of people, important to have 'real' microbiologists." (Policy analyst)

"Mix of speakers - different backgrounds and perspectives. Lots of discussion time. Clear aims, good construction of agenda." (NGO)

"The speakers mix" (Synthetic biologist)

"Diversity of contributors" (Regulator)

"Open discussion" (Microbiologist)

"Very interesting talks plenty of time for discussion. Very interesting and useful discussion." (Synthetic Biologist/Microbiologist)

"Structure" (Synthetic biologist)

"General forum to bring together scientists from different fields, regulators, NGOs with significant expertise in a very constructive atmosphere; no agenda for discussion - free development." (Regulator/Microbiologist)

"The willingness of different groups to listen to each other" (Social scientist)

"Time for discussion in addition to formal talks" (Microbiologist)

"Respectful, open discourse." (Research funder)

"Level of discussion between participants, which was excellent and informative" (Synthetic biologist)

Other comments received

"Of all the workshops on Synthetic Biology I have attended, I think yours was one of the most productive. I think that the case studies, especially the one on arsenic detection, might have been better chosen, but that said, it think they helped draw out the major questions." (NGO)

"I found the day extremely interesting and it was a pleasure and privilege to be involved. It was a very unusual mix of disciplinary positions and orientations with respect to the politics of synbio. Yet the evidently careful thinking about who was involved on what basis (and in what sequence) helped to build a conversation that – whilst addressing some of the most tricky issues – avoided the usual barricades. I particularly valued the conversations that took place in the wing and afterwards. Although these divergent perspectives present persistent challenges and there remains unreconciled diversity and contention, I felt that the workshop did go some useful way towards building greater mutual understanding. Since positive ways forward rest more on the mutually respectful exploration of difference, than on the engineering of consensus, the workshop was a very useful further step." (Social scientist)

PROGRAMME FOR THE WORKSHOP

9:30-9:50 **Registration**

9:50-10:00 **Welcome and Introduction** – Claire Marris

10:00-11:00 **Session 1: Learning from past experiences with released GMMOs**
Chair: Tom Ellis
Penny Hirsch - GM *rhizobium* inoculants as biofertilisers (15 minutes)
Ian Thompson - Field release of free-living GM bacteria (15 minutes)
DISCUSSION (30 minutes)
Key questions: *Why did genetically modified micro-organisms (GMMOs) aimed at tackling environmental and agricultural challenges fail to develop into successful products in the 1990s?*
And how might synthetic biology overcome those challenges?

11:00-12:30 **Session 2: Regulation for contained use and deliberate release of GMMOs**
Chair: Ollie Wright
Mark Segal - US regulations (15 minutes)
Martin Cannell - UK/EU regulations (15 minutes)
Hauke Harms - ARSOlux experiences (15 minutes)
DISCUSSION (45 minutes)
Key questions: *How do regulations in the USA, the EU and the UK distinguish between and deal with deliberate release and contained use of GMMOs?*
How might this evolve if synthetic biology is successful in producing a spectrum of applications that involve GMMOs that require deliberate release to perform their function?
How do local social and institutional factors blur the regulatory distinction between deliberate release and contained use; and how should this affect decision-making?

12:30-13:30 **LUNCH**

13:30-14:30 **Session 3 - Horizontal gene transfer and engineered biocontainment**
Chair: Catherine Jefferson
Ollie Wright - Built-in biosafety design (15 minutes)
Pascal Simonet - Horizontal gene transfer in the environment (15 minutes)
DISCUSSION (30 minutes)
Key questions: *What engineering approaches to biocontainment have been proposed by synthetic biologists and to what extent do they provide solutions to biosafety concerns?*

14:30-15:30 **Session 4 - Broader perspectives on risk and uncertainty**
Chair: Guy-Bart Stan
Ricarda Steinbrecher - Perspectives from an environmental NGO (15 minutes)
Andy Stirling - Risk, uncertainty and the governance of technology (15 minutes)
DISCUSSION (30 minutes)
Key questions: *What are the limitations of current governance frameworks for dealing with risk and uncertainty and how could they be addressed?*

15:30-16:00 **BREAK**

16:00-17:30 **General discussion**
Chair: Claire Marris

17:30 **END**

17:30-19:30 **Reception**

Aim:

To explore the prospects for synthetic biology applications for environmental and agricultural purposes that would require the intentional use of engineered micro-organisms outside of laboratories and large-scale industrial installations, including issues related to regulatory appraisal, environmental risk assessment, and risk reduction measures proposed by synthetic biologists.

Objectives:

1. Explore past experiences with the deliberate release of engineered micro-organisms for environmental and agricultural purposes from the period 1990-2005, when there was much hope in this area.
2. Generate a better understanding among UK synthetic biologists of existing UK/EU and US risk regulations that currently apply to synthetic biology applications for both 'contained use' and 'deliberate release' of genetically modified micro-organisms into the environment.
3. Explore how existing UK/EU/US regulatory frameworks would apply to synthetic biology applications involving engineered micro-organisms that perhaps fall in between strict 'contained use' and full blown 'deliberate release'.
4. Explore the potential contribution to risk reduction of technical approaches using synthetic biology for 'biological containment' to prevent horizontal gene transfer.
5. Promote a constructive discussion on these issues between different actors, including synthetic biologists, scientists from other relevant fields, regulators, social scientists and environmental NGOs.

Format:

Participation in the workshop was by invitation only, and numbers were kept low to allow for productive interactions. A scoping report was produced and circulated in advance to serve as the basis for discussion.
The workshop was be held under the Chatham House Rule and a report summarising the discussions was published online.

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1. The project "An Infrastructure for Platform Technology in Synthetic Biology" led by Prof. Richard Kitney at Imperial College London and involving researchers from Imperial, King's College London, Edinburgh University, Cambridge University and Newcastle University, who together form the Flowers Consortium, funded by the Engineering and Physical Sciences Research Council (EPSRC).
http://gow.epsrc.ac.uk/NGBOViewGrant.aspx?GrantRef=EP/J02175X/1

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LIST OF PARTICIPANTS

Jim Ajioka	Dept. of Pathology, Cambridge University
Geoff Baldwin	CSynBI, Division of Molecular Biosciences, Imperial College London
Travis Bayer	CSynBI, Division of Molecular Biosciences, Imperial College London
Jane Calvert	Science Technology and Innovation Studies, University of Edinburgh
Martin Cannell	GM Team UK Government Department for environment, food and rural affairs (Defra)
Belinda Clarke	Lead Technologist, Synthetic Biology, Technology Strategy Board
Janet Cotter	Greenpeace International Science Unit, Exeter University
Vasiliki Flari	Food and Environment Research Agency (FERA)
Chris French	School of Biological Sciences, Edinburgh University, Flowers Consortium
Michele Garfinkel	European Molecular Biology Organization (EMBO), Science Policy Programme
Jaydee Hanson	International Center for Technology Assessment, Washington D.C., USA
Hauke Harms	ARSOlux, Helmholtz Centre for Environmental Research, Leipzig, Germany
Penny Hirsch	Agroecology, Rothamsted Research
Kevin Martin	Porton Down, Defence Science and Technology Laboratory (Dstl)
Birgit Schönig	Federal Office for Food Safety and Consumer Protection, Berlin, Germany
Mark Segal	US Environmental Protection Agency, Washington D.C., USA
Pascal Simonet	Environmental Microbial Genomics Group, Ecole Centrale de Lyon, France
Ricarda Steinbrecher	Econexus
Andy Stirling	SPRU- Science and Technology Policy Research, Sussex University
Ian Thompson	Dept. of Engineering Science, University of Oxford
Brian Wynne	Cesagen, ESRC Centre for Economic and Social Aspects of Genomics, Lancaster University

Organisers:

Tom Ellis	CSynBI, Dept. of Bioengineering, Imperial College London
Catherine Jefferson	CSynBI, Dept. Social Science, Health and Medicine, King's College London
Claire Marris	CSynBI, Dept. Social Science, Health and Medicine, King's College London
Guy-Bart Stan	CSynBI, Dept. of Bioengineering, Imperial College London
Ollie Wright	CSynBI, Dept. of Bioengineering, Imperial College London