KING'S College LONDON

King's Research Portal

Document Version
Early version, also known as pre-print

Link to publication record in King's Research Portal

Citation for published version (APA):
Marris, C., & Jefferson, C. (2013). *Workshop on "Synthetic biology: containment and release of engineered micro-organisms" held on 29 April 2013 at King's College London: Scoping Report*. (N/A ed.) King's College, London.

Citing this paper
Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights
Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

•Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
•You may not further distribute the material or use it for any profit-making activity or commercial gain
•You may freely distribute the URL identifying the publication in the Research Portal

Take down policy
If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 06. Oct. 2023

KING'S College LONDON

Department of Social Science, Health & Medicine www.kcl.ac.uk/sshm

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

held on 29 April 2013

at King's College London

SCOPING REPORT

Claire Marris and Catherine Jefferson

July 2013

CSynBI

Centre for Synthetic Biology and Innovation

EPSRC

Engineering and Physical Sciences Research Council

Table of Contents

Table of Contents 1
Acknowledgements 2
List of Acronyms 3
1. Introduction 4
2. Past experience with GMMOs released into the environment 10
3. ARSOlux living biosensor case study 14
4. "Contained use" and "deliberate release": blurred boundaries 19
5. Built-in biocontainment for biosafety 22
6. Broader perspectives on risk and uncertainty 24
7. Conclusion 25
Appendix 1: Definitions of GMOs in UK legislation 26
Appendix 2: Definition of GMOs in EU legislation 27
Appendix 3: Definition of Intergeneric Microorganism in US Coordinated Framework for Regulation of Biotechnology 28
Appendix 4: Procedure to exclude a GMMO from the scope of Directive 2009/41/EC on contained use 29
References 30

List of Boxes and figures

Box 1: Examples from the Bayer Lab at Imperial College London of synthetic biology applications that would involve the deliberate release of GMMOs into the environment 7
Box 2: Whole-cell arsenic biosensor project funded by the Wellcome Trust 8
Figure 1: Flow chart for the EU/UK regulatory process relating to the deliberate release of GMMOs 9
Figure 2: Number of notified experimental releases of GMMOs in the EU per year (1991-2012) 11
Box 4: UK and EU Regulatory definitions of "contained use" and "deliberate release" 19
Figure 3: The six steps in Environmental Risk Assessment 24

Acknowledgements

The authors would like to thank all those who assisted with this report and shared their time and expertise, whether via correspondence, during interviews or by providing feedback on earlier drafts: Jim Ajioka, Travis Bayer, Martin Cannell, Sarah Carter, Janet Cotter, Tom Ellis, Chris French, Michele Garfinkel, Catherine Golstein, Sonja Hahn-Tomer, Penny Hirsch, Eric Hoffman, Jaydee Hanson, Todd, Kuiken Megan Palmer, Helena Paul, Martin Rémondet, Birgit Schönig, Mark Segal, Pascal Simonet, Guy-Bart Stan, Ricarda Steinbrecher, Andy Stirling, Ian Thompson, Ollie Wright, Brian Wynne. The contents of this report do not necessarily reflect their views and responsibility for views, omissions or errors remains with the authors.

We also thank the Engineering and Physical Sciences Research Council (EPSRC), the Defence Science and Technology Laboratory (DSTL), the Biotechnology and Biological Sciences Research Council (BBSRC) and the Medical Research Council (MRC) for their financial support.

This report should be cited as:
Marris, C. and Jefferson, C. (2013). "Workshop on 'Synthetic biology: containment and release of engineered micro-organisms' held on 29 April 2013 at King's College London: Scoping Report".

This report, as well as the report summarising the discussions at the workshop, and the slides from the workshop 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

List of Acronyms

ACRE	Advisory Committee on Release to the Environment (UK)
Defra	Department for Environment, Food and Rural Affairs (UK)
DNA	Deoxyribonucleic acid
EAEM	European Agency for the Evaluation of Medicinal Products
EC	European Commission
EFSA	European Food Safety Authority
ERA	Environmental Risk Assessment
EU	European Union
GEOs	Genetically engineered organisms
GM	Genetically modified
GMM	Genetically modified microorganism
GMMOs	Genetically modified micro-organisms
GMOs	Genetically modified organisms
HGT	Horizontal gene transfer
HSE	Health and Safety Executive (UK)
iGEM	International Genetically Engineered Machine competition
NGO	Non-governmental organisation
OECD	Organisation for Economic Co-operation and Development
rDNA	Recombinant DNA
SACGM	Scientific Advisory Committee on Genetic Modification (UK)
SMOs	Synthetically modified organisms
SynBERC	Synthetic Biology Engineering Research Center
TNT	2,4,6-trinitrotoluene
UK	United Kingdom
UNICEF	United Nations Children's Fund
US	United States
WHO	World Health Organisation

1. Introduction

The aim of this report, and of the related workshop, is to explore the potential development and commercialisation of synthetic biology applications that would require the intentional use of genetically modified micro-organisms (GMMOs) outside of laboratories and large-scale industrial installations in order to perform their intended functions, and to explore issues related to risk regulation frameworks, risk assessment methodologies and proposed risk reduction measures for these applications. It aims to move beyond debates about the definition of synthetic biology, and beyond speculative discussions about the hopes and fears associated with the field, to empirically analyse the prospects for these proposed applications.

This report was compiled from:

1. a review of the academic literature in social, natural and engineering science and of grey literature reports;
2. a dozen exploratory interviews and additional email exchanges conducted in February and March 2013 with regulators, NGOs, and scientists; and
3. three years of participant observation in scientific and policy arenas where synthetic biology is discussed.

This report set down the foundations for the discussions held at the Workshop on "Synthetic biology: containment and release of engineered micro-organisms" that took place at King's College London on 29th April 2013, and brought together synthetic biologists, microbial ecologists, environmental NGOs, regulators, social scientists, policy analysts, and research funders. The workshop aimed to:

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; and
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.

The majority of the current research in synthetic biology uses micro-organisms (bacteria, yeast or algae) as the host cells (or "chassis"), and the first wave of commercial applications emerging from synthetic biology is likely to consist of natural compounds produced by micro-organisms in industrial fermentation facilities. A few examples are already on or near the market, for example: artemisinin, a therapy for malaria (Sanofi, 2013), squalene, a moisturizing ingredient used in many cosmetics (McDougall, 2011), and a number of enzymes, fragrances and food flavourings (Synthetic Biology Project, 2012). The ETC group has produced a useful series of technology assessment case studies analysing the potential impacts of products of this kind (artemisinin, squalene, Star Anise, Vanilla, Safron and Vitiver oil) on livelihoods and biodiversity[1].

This report focuses on a different set of applications that would require the intentional release of genetically modified micro-organisms into environments beyond laboratories and factories *in order to perform their function*. These include applications in agricultural, environmental, energy and mining sectors, and the most commonly cited examples are:

1. Agricultural: biofertilisers, biocontrol of plant diseases and plant pests, delivery of growth promoting factors (e.g. auxin)
2. Environmental: biomonitoring and/or bioremediation of environmental pollutants (e.g. heavy metals) in drinking water or soil; or detection of molecules of the explosive TNT

[1] Available at http://www.etcgroup.org/content/case-study-artemisinin (all the websites cited in this report were last accessed on23/07/2013).

3. Mining: biomining/bioleaching of metals
4. Energy: use of algae, or consortia of algae and bacteria, to produce biofuels and/or high value compounds in outdoor ponds
5. Industry: use, modification or eradication of bacterial biofilms that coat pipes to modify their surface properties

A number of research projects currently underway within the Flowers Consortium[2] aim to develop such products (see Boxes 1 and 2 for some examples). Some more speculative and playful applications have also been proposed by teams competing in iGEM[3], such as the 2010 Cambridge iGEM team's consideration of bioluminescent bacteria in trees to replace conventional street lighting. Overall, a significant number of the applications envisaged by iGEM projects would require deliberate release. For example, of the 191 teams registered for iGEM 2012, at least 15 of the projects involved some form of bioremediation, ranging from degradation of plastics in the marine environment (UCL) to removal of pollutant molecules that adhere to the surface of statues (Trento). The AuxIn project[4] (2011, Imperial College) that aims to combat desertification, recently heralded at a conference on synthetic biology and conservation (Yong, 2013), is another example.

There is also a category of applications using GMMOs where release into the environment is likely but not necessarily intentional. These include most of the GMMOs intended for medicinal and veterinary purposes, and also compounds produced by GMMOs in fermenters but not fully purified before use, such as the local production of genetically modified yeast to produce strigolactone envisaged in Box 1. GM algae grown outdoors in ponds and/or plastic tubular photobioreactors are also considered to be likely to lead to localised releases, due to failures and leakages.

Finally, there is a category of envisaged products that are intended for use outside of laboratories and factories, but that do not need to be released into the natural ecosystems to perform their function. These are whole cell living biosensors contained in sealed vials, intended for use in the field. ARSOlux is a whole cell biosensor for detection of arsenic in drinking water that is ready for market and will be discussed further in Section 3; and two whole cell biosensors intended for field use are also being developed within the Flowers Consortium. The first is another arsenic biosensor that started off as an iGEM project in 2006 at Edinburgh University and is now a Wellcome Trust funded project led by Prof. Jim Ajioka at Cambridge University (see Box 2 and Bailey, 2013); and the second is a biosensor to detect proteases released by parasites such as *Schistosoma*, which started off as the "Parasight" Imperial College iGEM project of 2010[5], and is now being developed further by researchers at Imperial College with funding from the Bill & Melinda Gates Foundation.

As we shall see in this report, there are blurred boundaries between "contained use" and "deliberate release" of GMMOs, and these boundaries are likely to be further challenged if and when the GMMO applications envisaged by synthetic biologists for environmental, agricultural and mining uses enter the regulatory system, because those applications cover a whole spectrum in terms of the nature, scale, and time-horizon of the release (adapted from French et al., 2011):

1. micro-organisms used outside of the laboratory or factory, that use both physical and biological containment measures (e.g. field use of whole cell living biosensors in sealed vials);
2. micro-organisms released into the environment but intended to survive for only a few hours (e.g. whole cell bioreporters for TNT or environmental contaminants);

---

[2] The Flowers Consortium is made up of research teams from five UK universities that carry out research into synthetic biology: Imperial College London, Cambridge University, Edinburgh University, Newcastle University and King's College London. This report and the related workshop are being conducted under the auspices of the Flowers Consortium.

[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 (Zhang et al, 2011). Based on a kit of biological parts, teams work over the summer to design and build biological systems and operate them in living cells. For further information, see: www.igem.org

[4] http://2011.igem.org/Team:Imperial_College_London

[5] http://2010.igem.org/Team:Imperial_College_London

3. micro-organisms used in small-scale decentralised bioreactors (e.g. for production of biofuels on farms) and algae grown outdoors in plastic tubular photobioreactors, that are not intended to be released but likely to be subject to leakages leading to localised unintended releases;
4. micro-algae and/or bacteria grown in large-scale outdoor open air ponds for the production of biofuels and/or high value compounds, which will spread by airborne spores or animal vectors;
5. micro-organisms intended to survive in the environment indefinitely or at least for long periods of time in order to perform their function (e.g. bioremediation, biofertilisers, biopesticides, the AuxIn project and long term environmental biosensors).

For each of these scenarios, different questions arise for risk assessment and different risk reduction measures can be envisaged. Moreover, it is not always obvious which of these applications would be defined as "contained use" or "deliberate release" in EU/UK regulations. This report seeks to begin to identify the challenges raised by these applications and ways to address them. This seems a worthwhile and timely endeavour given that such applications are frequently promoted as synthetic biology products that could address important environmental, agricultural and health global challenges.

Novelty of synthetic organisms?

There are fundamentally opposing views on the novelty of the field of synthetic biology and the novelty of the risks associated with it (Zhang et al., 2011), and especially the extent to which it differs from existing forms of genetic modification. Synthetic biologists, when emphasising the novelty of the field, suggest that novel genes, novel functions and novel organisms that have no natural counterpart will be produced. If this were the case, regulators and policy analysts agree that it would have implications for the current framework of GMO risk assessment, since it currently relies on comparisons with natural counterparts for the "donor", the "recipient" and the genetic material transferred (Rodemeyer, 2009). Thus in 2007, the UK's Health and Safety Executive (HSE) appeared convinced of the capacity of synthetic biology to produce organisms novel enough to challenge the "precision" of risk assessment and suggested that this warranted higher containment measures (HSE, 2007):

> *Given the goal of synthetic biology is to create novel microorganisms, many of the risks associated with such activities will be complex, indefinable and difficult to anticipate with any degree of precision... Therefore, work of this type is likely to attract higher containment measures than would otherwise be applicable to the organisms on which they are based, or from which the genetic material has been derived.*

In 2008, however, the BBSRC convened a meeting with regulators, including the HSE, to discuss synthetic biology, which concluded that "none of the questions and hypothetical scenarios suggested a paradigm shift that would necessitate amending the UK's regulatory framework" and that "virtually all envisaged synthetic biology applications would fall within the GMM contained use regulations/EC Directive 2001/18/EC on the deliberate release of GMOs into the environment" (BBSRC, 2008). A few years later, the US Presidential Commission for the Study of Bioethical Issues and the UK Health & Safety laboratory came to similar conclusions (Bailey et al., 2012; US Presidential Commission for the Study of Bioethical Issues, 2010). Thus, while the novelty and revolutionary nature of synthetic biology is emphasised in some contexts, and seems to imply that something needs to be done, when examination of the regulatory agenda becomes more specific there always seem to be arguments that nothing else needs to be added (Zhang et al., 2011, p. 7).

Different terms have been used to describe the micro-organisms produced by genetic engineering techniques, including "genetically engineered organisms" (GEOs), "genetically modified organisms" (GMOs), and "genetically manipulated organism" (also abbreviated to GMO and used for a short period in UK and EU regulatory arenas in the 1980s). The term "transgenic" is also used, but usually for plants and animals rather than micro-organisms. With the advent of synthetic biology, the terms "synthetic organism" and "Synthetically Modified Organism" (SMO) are now emerging. In terms of environmental risk regulation, the most relevant terms are "genetically modified organism" (GMO) and "genetically modified micro-organism" (GMMO or GMM), used in UK and EU legislation, and "intergeneric microorganism", used in US regulation. The precise legislative definitions of what

counts as a "GMO" or "intergeneric microorganism" have important consequences for the way in which specific organisms are dealt with by regulatory frameworks (see Appendices 1, 2 and 3). Indeed, in the UK/EU system (and also to some extent in the USA), this is what determines whether or not the product enters the regulatory process at all (see Figure 1); and it is generally accepted that all micro-organisms likely to be produced by synthetic biology in the near to medium term fall within these definitions (Bailey et al., 2012; BBSRC, 2008; HSE, 2007; Pauwels et al. 2013; and Rodemeyer, 2009).

For the purposes of this report and workshop, we will consider all genetically modified micro-organisms, regardless of whether or not the process used to produce them has been labelled as synthetic biology, and will use the acronym GMMO to refer in a broad sense to all such organisms. The definitional issue will only be considered insofar as differing interpretations of synthetic biology's novelty could impact upon its treatment under regulatory and risk assessment regimes.

Box 1: Examples from the Bayer Lab at Imperial College London of synthetic biology applications that would involve the deliberate release of GMMOs into the environment
(Source: Travis Bayer)

a) Production of strigolactones for eradication of a parasitic weed in Africa

The parasitic witchweed species of the *Striga* genus are among the major biotic stresses on crop yield in Africa, affecting staples such as maize, sorghum, rice, and cowpea. *Striga* seeds lie dormant in soil until they detect the plant hormone strigolactone, which induces seed germination and attachment of the weed to host roots. The application of strigolactone to farmland before planting can induce "suicidal" germination of *Striga* seeds. However, the high cost of chemical synthesis of strigolactone has precluded the use of this strategy in the field. Synthetic biologists at the Bayer lab have engineered yeast to produce strigolactones and precursor compounds by introducing a synthetic metabolic pathway of plant and microbial origin. They found that microbially produced deoxystrigol is effective at germinating seeds of *Striga gesmonoides* at picomolar concentrations and suggest that this potentially low-cost microbial production of strigolactones could be used in a program of *Striga* eradication from arable land in Africa.

The delivery of strigolactones to the field could take several different forms. In one version, the compound is produced in a laboratory fermenter, extracted and packaged, and then delivered to fields where it is needed (methods of application to soil and discussions of farmer uptake and use are ongoing research areas). The advantage of this "extraction" option is that quality control during production can be implemented, and there is minimal risk of release of genetically modified organisms into the environment. The major disadvantage is the added cost of solvent extraction and transport. An alternative would be the "local" option, where strains of yeast are distributed to trained local users of the technology, who then produce the compound in a process similar to brewing in communities where it is needed. The strigolactone would then be applied by spreading the spent brewing media (either with or without yeast cells) on fields. The major advantage is greatly reduced costs (transportation and extraction) and the ability for local control of the technology. The disadvantage is the likely release of the genetically modified yeast.

b) Enhanced mining of copper using engineered bacteria

The extraction of copper from high grade copper-bearing ores is currently accomplished by a process termed *flotation*, which requires significant inputs of energy. An alternative is the use of *heap leaching*, where the activity of naturally occurring bacteria serves to solubilize copper sulphides (via the oxidation of ferrous iron in ores such as chalcopyrite). Heap leaching also allows the use of low grade ores (i.e. ores that have < 1% copper by mass), that are currently waste products in mining or excavation processes. The major limitation on heap leaching productivity is that the iron oxidation metabolism of the leaching bacteria is relatively slow and inefficient. In this project, we are developing methods to enhance this metabolic activity using genetically engineered constructs. The aim is to apply the engineered strains to industrial leaching operations. At production scale, a chalcopyrite heap can be on the order of 30 meters high, and several kilometres in length and width. Current heap leaching operations have developed methods to capture runoff (indeed this is where the solubilized copper is recovered), however, the operation occurs in the open air, presenting a deliberate release of the genetically modified bacteria on a massive scale. It should also be noted that the bacteria are unlikely to survive outside of the leaching environment, as they grow in acidic conditions (pH from 1 to 2).

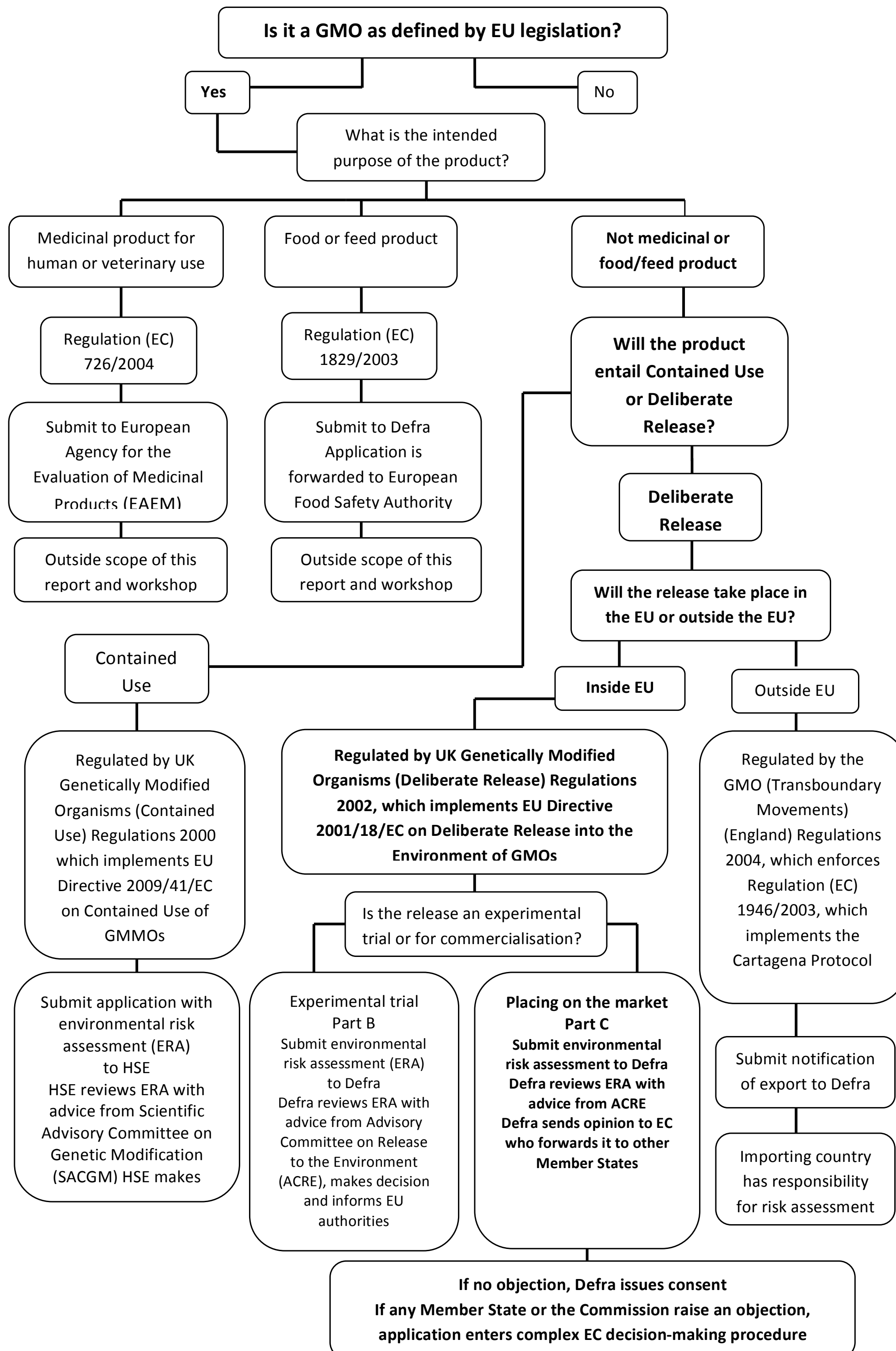
Box 2: Whole-cell arsenic biosensor project funded by the Wellcome Trust[6]
(Source: http://www.wellcome.ac.uk/Funding/Technology-transfer/Funded-projects/enabling-technology/index.htm)

Arsenicosis from chronic consumption of contaminated ground water affects virtually all organs and tissues where skin lesions, bronchitis, gastroenteritis and ultimately a range of cancers are typical pathologies.

Although arsenic contamination of drinking water is a global problem, it most seriously affects on the order of 100 million people in some of the poorest regions on earth including India/West Bengal, Bangladesh and Nepal. Prof James Ajioka's team at Cambridge University and Prof French at Edinburgh University are aiming to construct an inexpensive and reliable kit to assess arsenic contamination in drinking water in rural villages. Based on the observation that some bacteria detect arsenic, they will engineer an arsenic sensing device based on the *Bacillus subtilis* arsenic operon. This biosensor will be combined with a reporter system based on the violacein operon, resulting in bacteria that would turn green when it detects very low, safe levels of arsenic in the drinking water, but if the arsenic contamination is at a dangerous level, it will turn violet. The transcriptional signal to drive the pigment device in the bacteria can be tuned to respond to arsenic levels within definition of WHO safe or dangerous levels. The kit will be based on a weakened strain of the harmless soil dwelling bacteria, *B. subtilis*, housed in a robust plastic container to further reduce any risk and for easy, environmentally friendly deactivation/disposal.

[6] For up to date information on this project, see: http://www.arsenicbiosensor.org

Figure 1: Flow chart for the EU/UK regulatory process relating to the deliberate release of GMMOs
(Source: compiled by authors from legislative texts)



2. Past experience with GMMOs released into the environment

The use of GMMOs in the agricultural, environmental and mining sectors has been discussed since the 1980s and it is interesting to note that most of the environmental and agricultural applications being discussed today for synthetic biology were already envisaged in the 1980s, for example in the 1989 report by the Royal Commission on Environmental Pollution (RCEP, 1989, p. 11):

> *Released organisms are also likely to be used in mining, for the recovery of heavy metals, in the control of oil spills, for water purification and in pollution control generally. Naturally occurring micro-organisms are already used for copper extraction, for example, and for degrading chemicals in toxic waste. New organisms may also be used in the longer term to generate alternative sources of fuel replacing conventional ones such as timber or oil and for the development of 'biosensors' to assist with control of industrial processes or for diagnostic use in medicine.*

The first experimental field releases of GMMOs were carried out in 1986, and at that time there were high hopes that a suite of products of this type would enter the market rapidly, with significant economic benefits (Hirsch, 2004; RCEP, 1989; Snow et al., 2005; Tiedje et al., 1989). However, 25 years on, hardly any commercial products consisting of a GMMO that requires deliberate release into the environment to perform its intended function have entered the EU or US market, very few experimental releases are currently being conducted, and firms seem reluctant to invest in this area. In this section, we explore the reasons for this apparent failure in order to investigate whether and how synthetic biology might overcome the problems encountered by similar product development in the 1980s and 1990s.

GMMOs developed in the 1980s and 1990s[7]

Research in the 1980s and 1990s focused on attempts to improve efficacy of symbiotic rhizobia, biopesticides, plant growth-promoting rhizosphere bacteria and microorganisms for bioremediation; and to demonstrate this in the field (Hirsch, 2004). In 1989, the company BioTechnica International conducted field tests in the USA for soya beans and alfalfa inoculated with *Sinorhizobium meliloti* and *Bradyrhizobium japonicum* genetically engineered to improve nitrogen fixation (Bosworth et al., 1994; Ronson et al., 1990). These field tests were approved by the US Environmental Protection Agency (EPA). Significant yield improvements were not obtained and the technology was not taken up commercially at that time. Limited commercialisation of an intergeneric microorganism *Sinorhizobium meliloti* by the company Research Seeds, Inc was approved by the EPA[8] in 1997 as a microbial seed inoculant coat for alfalfa seeds but this product does not appear to have met with commercial success.

In Europe, field releases were conducted to provide data for use in risk assessment of potential future applications of GMMOs (Hirsch, 2004). Examples include the testing of marker genes and containment strategies in Ireland in 1991 and Germany in 1995 (O'Flaherty et al., 1995; Selbitschka et al., 2006); and a *Rhizobium leguminosarum* genetically engineered to enable efficient selection and screening released in the UK in 1994, in an experiment to determine the frequency of acquisition of symbiotic genes by inoculant strains (Hirsch, 2004; Selbitschka et al., 1995). The outcome of these experiments was an understanding that containment strategies designed in the laboratory are likely to be less effective in the environment, probably due to the great variation in conditions, and that with sensitive selective markers, survival of GMMOs can be demonstrated at low levels for long periods of time (Hirsch, 2004).

In China, a plant growth-promoting rhizosphere bacteria capable of fixing nitrogen, *Alcaligenes eutrophus,* has been engineered to increase fixation and was approved for limited commercial use as a biofertilizer in Northern China (Huang, 2002).

---

[7] We thank Dr Penny Hirsch for her help with this section.

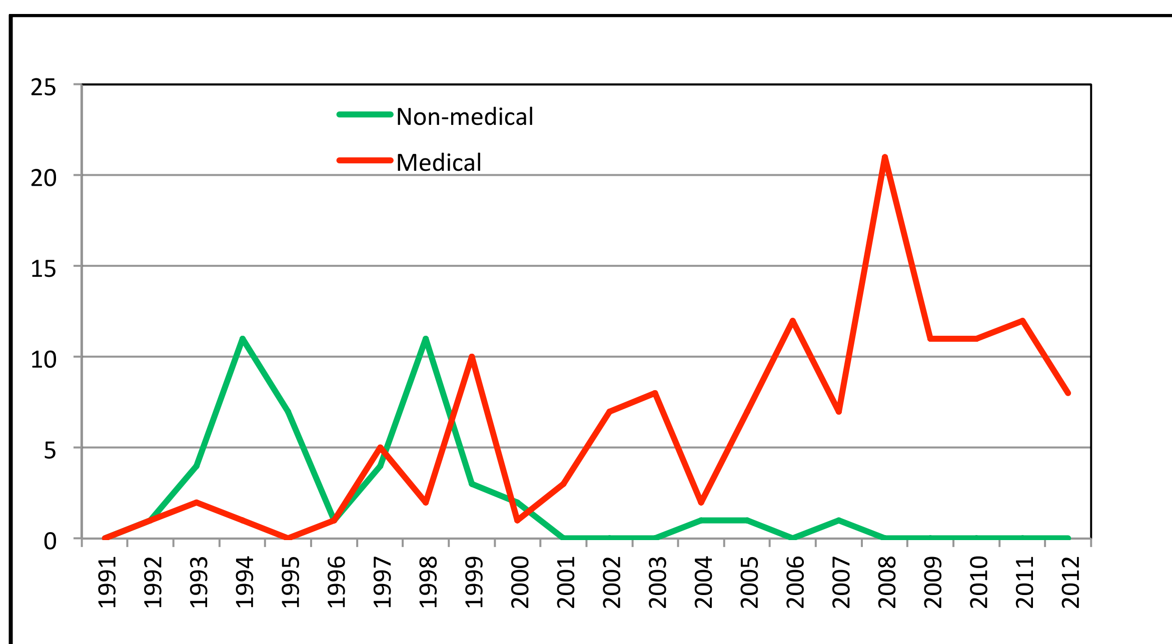
[8] http://www.epa.gov/biotech_rule/pubs/factdft6.htm

Overall, despite significant research efforts and hope invested in this area, this work has not led to a series of commercially available GMMOs products capable of improving nitrogen fixation and/or agricultural yields.

Dwindling number of experimental releases

After an initial wave of field trials in the period 1987-2000, the number of field trials for GMMOs (excluding those for medical and veterinary uses) has dwindled to almost nothing in the last decade (see Figure 2). There were 44 releases of non-medical GMMOs in the EU in the period 1991-2000, just 3 in the period 2001-2007, and none at all since then[9]. In the USA there have been even fewer experimental releases of GMMOs: 26 were conducted between 1998 and 2007, and none at all since then[10]. All of these trials were notified to national regulatory authorities, were subjected to risk assessments and obtained regulatory approval (information about releases that might have occurred without being notified to regulatory authorities is by definition difficult to obtain).

Figure 2: Number of notified experimental releases of GMMOs in the EU per year (1991-2012)
(Source: Compiled by the authors from the list of Summary Notification Information Formats - SNIFS - circulated under Article 9 of Directive 90/220/EEC and Article 9 of Directive 2001/18/EC, http://gmoinfo.jrc.ec.europa.eu/overview/doc/snifs.pdf)



Current reluctance of firms

We contacted a number of firms in the UK during the preparation of this report and workshop, seeking to identify firms who had already developed GMMO products involving deliberate release or were planning to do so. We acknowledge that firms will understandably be unwilling to disclose their commercial strategies, and will work on multiple potential strategies in parallel, but we did receive a consistent set of striking responses, with firms firmly stating that they currently had no intention of developing such products. They were happy to point to the use of GMMOs and of synthetic biology as a platform for the production of high value compounds in bioreactors, i.e. in "contained use" situations. However, when it comes to applications that would require deliberate release, firms appear

---

[9] The first notified experimental releases of GMOs in the EU took place in 1986 but data was not systematically collated before 1991.

[10] http://www.epa.gov/oppt/biotech/pubs/submiss.htm

reluctant to publicly acknowledge any significant interest or investment. Firms do already use micro-organisms widely for bioremediation, biomining and biocontrol, but seem to prefer using strains isolated from natural ecosystems (and perhaps improved via mutation and selection methods that do not involve recombinant DNA techniques) rather than GMMOs. For example Ian Thompson, Professor at Oxford University and co-founder of the bioremediation firm Microbial Solutions Ltd told us (email correspondence, 07/03/2013):

> *The company I co-founded, Microbial Solutions Ltd, only uses natural bugs. Our customers [which include large multinationals] would drop it as quickly as hot coal if they thought it was GM based. However, we do use GM biosensors – but have to make it very clear their application is solely in the lab.*

This reluctance of firms to endorse (or openly endorse) the prospects for GMMOs to be used beyond the confines of factories is in stark contrast to the enthusiasm exhibited by synthetic biologists for such applications.

Reasons given for the failure of the first wave of GMMOs intended for release

Four overlapping reasons are given by stakeholders to explain the lack of development of the products envisaged in the 1980s and 1990s:

1. **Viability of products**: producing GMMOs for environmental and agricultural applications turned out to be harder than expected and the GMMOs produced did not perform their intended functions effectively and reliably enough to be of commercial use. This in turn is understood to be due to either:

    a) the **complexity of natural microbial ecosystems and of their interactions with the environment**. This view is mostly expressed by microbial ecologists and by environmental NGOs.

    or

    b) the **lack of sophistication of the genetic engineering conducted so far** to produce GMMOs. This view is expressed by synthetic biologists, who emphasise that some of the efficacy issues of the past may now be addressed using synthetic biology approaches, but tends to be contested by more traditional molecular biologists and systems biologists who have not embraced synthetic biology as a radically new and promising field, and by microbial ecologists.

2. **Regulatory hurdles**: the time and expense necessary to comply with regulations for the appraisal of environmental and health risks (that are considered by some actors to be unnecessarily strict); and/or uncertainty about how these GMMOs will be considered by existing regulations, especially whether or not an application will be considered as "contained use" or "deliberate release"; or the lack of a clear regulatory framework in the country where the application is intended to be used.

3. **Negative public image of GMOs**: although there are contrasting understandings of the causes and dynamics of public controversies about GMOs, there is a general agreement among all stakeholders that GMOs (or at least GM crops) now have a negative public image (especially in Europe), and one that is "not green". This makes it difficult for companies that position themselves in the market as providing more environmental solutions than existing options to openly adopt GM solutions. This can apply for example to water treatment, bioremediation, biomining; and bacterial inoculants or seed coatings for increased plant growth and/or pest tolerance.

4. **Availability of adequate non-GM options:** naturally occurring micro-organisms (or organisms modified and/or selected without using rDNA techniques) can perform the same functions, possibly as effectively as or more effectively than GMMOs, more cost-effectively and with less regulatory oversight.

Position 1(a) is well exemplified by some microbiologists (e.g. Hirsch, 2004; Viebahnmet al., 2009). As an illustration, Professor Ian Thompson says (e-mail correspondence 07/03/2013):

> *Synthetic biology holds great potential in instances such as GM modification of* E.coli *for production of insulin for example. You have a pure culture in a bioreactor and get good production. However, the examples synthetic biologists use whereby the technology will revolutionise environmental applications, such as my expertise bioremediation, is unlikely to succeed for some considerable time. We did the first release of free-living GM bacteria in Oxford in the 90s - the GMO could not compete with the indigenous community and eventually died out. There is no way that synthetic biology can outbid 3.5 billion years of evolution, at least not until we know a lot more about microbial ecology and physiology.*

The contrasting position 1(b) is exemplified, for example, by de Lorenzo (2010, p. 926):

> *The term Genetic Engineering constitutes a strong metaphor that evokes the action of joining together otherwise separate components with given properties following a rational blueprint; but this time using genes as the building blocks of the system. In reality, the type of Genetic Engineering started by Stanley Cohen and Herbert Boyer in the early 70s was mostly, and for a long time (until the simplification of DNA sequencing techniques), a trial-and-error endeavour with very little rational design. Even when DNA sequences became prolifically available, such genetic manipulations depended, to a large extent, on the operator's abilities and were subject to considerable unpredictability. Synthetic Biology (at least one of its branches) improves upon the strong rational and prospective design of early Genetic Engineering with the use of bona fide engineering methodology rather than a simple analogy.*

A similar perspective is also provided by Wright et al. (2013, p. 1221):

> *the success of the Asilomar approach may not only be due to stringent GMM regulation and a subsequent limited number of environmental releases, but more because the effectiveness of engineered microbes has been poor. […] Synthetic biology, with a more holistic approach to cell engineering, appears poised to change this.*

One reason for the scepticism displayed by some actors in response to such declarations is that at the time when genetic engineering first merged, strong claims were also made by proponents of the field about the precision of the new techniques. Thus, molecular biologists in the 1980s emphasised the lack of sophistication of previous techniques to emphasise the enhanced predictability of the then new recombinant DNA (rDNA) techniques, as exemplified in the seminal OECD report of 1986 (OECD, 1986)[11]:

> *While rDNA techniques may result in the production of organisms expressing a combination of traits that are not observed in nature, genetic changes from rDNA techniques will often have inherently greater predictability compared to traditional techniques, because of the greater precision that the rDNA technique affords to particular modifications. […] It is acknowledged that additional research and experience with rDNA micro-organisms, plants, and animals, should certainly increase our ability and precision to predict the outcome of introductions of rDNA organisms into the many varied ecosystems.*

Despite these claims of greater predictability and precision, studies have now found that unintended additional fragments of DNA are often present (Rang et al., 2005; Windels et al., 2001), and that unintended re-arrangements of sections of the host genome flanking the genetic insert were present in GM crops that have been placed on the market (Hernández et al., 2003; Windels et al., 2001). Also, a recent study by scientists from the European Food Safety Authority demonstrated that some long versions of the cauliflower mosaic virus 35S promoter, very commonly used in genetic constructs and present in a number of GM crops on the market, unexpectedly contain an overlapping viral open reading frame which, when expressed, might result in unintended phenotypic changes (Podevin and du Jardin, 2012). This demonstrates that even seemingly well-characterised and routinely used non-coding regulatory DNA sequences can produce unpredicted, unintended effects, yet such sequences are commonly assumed to present little risk when assessed within the EU regulatory framework, and "intergeneric microorganisms resulting only from the addition of well-characterized, non-coding regulatory regions" are excluded from the US Biotechnology Coordinated Framework.

[11] Note also how similar the definition of biotechnology given by the OECD in 1982 is to some current definitions of synthetic biology: "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (Bull et al., 1982).

Opinions among scientists and other stakeholders are sharply divided about whether any future forms of genetic engineering, including synthetic biology, could ever result in the creation of organisms with reliably predictable behaviours, especially when released into complex, diverse and variable natural ecosystems; and this is directly related to divergent evaluations of the prospects for GMMO products that would need to be released into the environment in order to perform their intended function.

Different explanations for past failures lead to different proposals for future solutions

Exploring the reasons for the past failure of GMMOs intended for release is important, because depending on how that failure is understood, different solutions will be proposed for future developments in this area. For example, actors who believe that the explanation for the past failure in the development of these GMMOs lies in mismanaged public fears rooted in lay people's misunderstanding of science will tend to focus their attention on opinion polls and initiatives for science communication aimed at reassuring the public. If, however, the explanation is understood to lay in the complexity, diversity and dynamic nature of natural ecosystems, and in scientists' inability to produce organisms that behave predictably and reliably in those environments, then a possible avenue might lie in seeking to collaborate more closely with scientists in other fields who know more about those ecosystems.

The latter stance could also lead to the conclusion that a certain level of "containment", even for GMMOs deliberately released into the environment, could be helpful as a way to make the products more viable, rather than as a safety or public trust generating strategy. From this perspective, GMMO applications designed to work in a specific circumscribed environment for a shorter defined period of time are more likely to perform their intended function effectively than those designed for full blown, broader and long term dissemination into the environment. For example GMMOs may be more effective for bioremediation in industrial waste streams (captured before they leave an industrial site); or they could be released in a small area in which an essential nutrient required for their growth has been provided externally. The reason for this can be interpreted as reducing regulatory hurdles or negative public reactions, but it is also proposed as a way to ensure more control of the environment in which the organisms are used, and thus to improve their efficiency and reliability.

3. ARSOlux living biosensor case study[12]

A bacterial arsenic biosensor known as ARSOlux has been developed by researchers at the publicly-funded Helmholtz Centre for Environmental Research (UFZ) in Leipzig, Germany. By 2010, ARSOlux was a technically well-developed, field-tested and patented product and the ARSOlux-team was formed, with the objective of launching ARSOlux on the market. The biosensor consists of whole live cells of the bacteria *E.coli* strain K12, which has been genetically engineered to emit light when brought into contact with arsenic dissolved in water. The arsenic-induced light intensity (luminescence) is detected optically by a portable measuring device (luminometer), which allows for precise quantification of the concentration of arsenic in water samples. The living cells are freeze-dried in sealed vials and usable for up to three months at room temperature. The stated aim of the developers is to provide an easy to handle kit to test drinking water in the field, which is more environmentally friendly and cheaper than chemical methods of arsenic detection currently used.

The first field tests were conducted in 2005 in Vietnam. The ARSOlux-team analysed samples from river deltas and demonstrated comparable accuracy to chemical methods of testing (Trang et al., 2005). In 2010, a field campaign was conducted in Bangladesh to perform further tests and to explore the potential market. Knowledge was gathered about the usability of the biosensor considering locally challenging geographic, geologic and climatic conditions. The team reported that field measurements were accurate; that their bioreporter performed better than two commercial chemical tests in terms of practicality, material requirements and waste disposal; and that a higher number of tests could be conducted within a day. Furthermore, they observed variable levels of arsenic contamination in

[12] Sources: ARSOlux Press Release, October 2010, https://www.ufz.de/index.php?en=20722; ECO-India Project webpage, http://www.eco-india.eu/; UFZ, Webpage of ARSOlux Projects, http://www.ufz.de/arsolux/index.php?en=20707.

nearby wells from the same village, thereby suggesting that switching wells could be an effective strategy to avoid high arsenic exposure (Siegfried et al., 2012, p. 3281).

The team, that included a geographer and an economist as well as microbiologists, also spoke to villagers, government officials, and experts working for the World Health Organisation (WHO) in Bangladesh to better understand the issues at stake. The team learnt that despite arsenic water contamination being a major health problem, adopting new methods for arsenic detection is not at the top of the Bangladeshi government's agenda, compared, for example, to large-scale floods and food shortages. While the field tests provided valuable data on the usability and reliability of the device in real world situations, obtaining support and approval from the Bangladeshi government for the commercial deployment of the device has proved to be a more difficult problem. The lack of a clear regulatory framework for GMOs and the political instability in the country have further exacerbated the challenges faced by the ARSOlux team.

In order to demonstrate the safety of the device and their willingness to comply with regulatory authorities, the team decided to seek approval for field tests of ARSOlux in Germany. When the application for this trial was submitted to the relevant German authorities, discussions occurred about whether this application should be considered as "contained use" or "deliberate release", in order to determine which regulatory framework should be applied. The conclusion reached was that the field test would not be considered as "deliberate release" and should thus be regulated under German legislation that enacts EU Directive 2009/41/EC. This decision was reached on the basis that the proposed field test fell into the definition of "contained use" in Directives 2009/41/EC and 2001/18/EC, because it uses "specific containment measures" "to limit the contact [of the GMO] with and to provide a high level of safety for, the general population and the environment". These measures included the relatively robust and sealed glass tube, and characteristics of the vector-recipient system that are formally recognised as "biological safety measures" specifically listed in the German Genetic Engineering Safety Regulation which specifies the requirements of the German Genetic Engineering Act of 1990 (see Box 3)[13].

[13] We thank Birgit Schönig from the BVL for her valuable input into this paragraph.

Box 3: Vector-recipient systems recognised as biological safety measures in the German Genetic Engineering Safety Regulation

Article 6 in connection with Appendix II of the German Genetic Engineering Safety Regulation

The following conditions must be met:

Recipient organism:

- replication only under conditions that are not, or seldom found outside of genetic engineering installations
- no disease inducing properties in humans, animals or plants and no hazards to the environment
- low horizontal exchange of genes with animal or plant associated organisms.

Vector:

- an existing limited host specificity; and
- no self transfer system, low co-transfer rate and low mobilization ability; or
- not infectious in itself and low transfer by endogenous helper viruses.

What are the advantages of using of biological safety measures?

- waste and wastewater treatment activities can be reduced;
- by using biological safety measures the ascertained hazard potential of the GMO can be assessed as lower; and
- the genetic engineering operations can be assigned to a lower biosafety level.

Article 5 para. 5 and Article 7 para. 5

Examples of biological safety measures are:

- Escherichia coli K12 and pBR322
- Saccharomyces cerevisiae (haploid) and YEp
- Cell line Hela and pCDM8

It is also important to note that, in order to be defined as "contained use", the device could only be used in a vehicle equipped and licensed as an appropriate premise to conduct work with GMOs (a lab-in-a-van, see photo), and could only be used by a qualified scientist, supervised by a team leader with responsibility for ensuring that biosafety measures, including Good Laboratory Practice, were followed. This demonstrates how containment is not and cannot ever be defined solely in terms of physical and biological measures; a point that we return to in the next section.

This experimental field trial was conducted in 2011 in Saxony. ARSOlux have not applied for the authorisation to commercialise the device in Germany and have begun to look at potential markets in other countries in the Himalayan region where arsenic contamination in tube wells used for drinking water is a health problem. They are partners in the EC funded project ECO-India, which aims to develop cost-effective solutions for community-based water and wastewater treatment systems. In October and November 2011, the ARSOlux-team conducted workshops in Nepal and India, which brought together local government officials, local scientists, local companies and experts from local NGOs and large international NGOs such as UNICEF and WHO, to discuss current arsenic mitigation procedures, and how ARSOlux might be implemented locally.

Eight years after the demonstration of proof-of-concept for ARSOlux, and despite benefits that seemed obvious to the developers and a relatively simple case with respect to containment and environmental risk assessment, the product is still not on the market. This case study exemplifies a number of points that apply more generally, and that the ARSOlux team has over time begun to grapple with:

- Scientists (especially in academia) sometimes develop clever technologies and then search for real-world problems to solve, rather than starting from a serious real-world problem and searching for the best approach to address it (which may turn out to involve social, political and economic measures as much as scientific or technological innovation).

- When investigating how a new technology might address a real-world challenge, interaction with a wide range of stakeholders, early on in the process, is crucial in order to better understand the problem and how the proposed technology might fit into real-world situations.
- These discussions and ensuing decisions need to take into account existing and potential alternative measures to tackle the same problem (in this case existing chemical options and bringing water to a laboratory for testing).
- Lack of a clear regulatory framework, as in Bangladesh, can impede the commercialisation of a new technology.
- Containment measures for GMMOs include social and institutional measures as well as physical and biological ones (discussed further in next section).

Potential alternative regulatory strategy for whole cell living biosensors?

The UK synthetic biology research team developing a bacterial whole cell living biosensor, led by Prof Jim Ajioka at Cambridge University (see Box 2) is facing a similar quandary with regards to the demarcation in the EU regulatory system between contained use and deliberate release. Following meetings with HSE and Defra, the team is considering applying for the GMMO used in their device to be exempt from Directive 2009/41/EC on the contained use of GMMOs, on the basis that it meets the criteria established in Annex II, Part B of the Directive "establishing the safety of the GMMs for human health and the environment" (see Appendix 4 of this report for details). GMMOs that meet these criteria can in principle be added to a list in Annex II, Part C of the Directive, on a case-by-case basis, and are then excluded from the scope of the Directive.

In order to go down this route, two challenges will need to be faced. Firstly, the regulatory procedure to add a GMMO to this list would involve all EU Member States. A draft measure would be submitted to the European Commission's regulatory committee, which would then issue an opinion before the measure is subjected to voting by Member States, according the usual quality majority vote (as set out in Article 205(2) of the Treaty establishing the European Community). No one has yet sought to apply for such an exemption, so Part C of Annex II of Directive 2009/41/EC is currently still blank, and indeed the regulatory committee to implement this procedure has not even been established.

Secondly, such an exclusion "applies only when the GMM is used under conditions of contained use as defined in point (c) of Article 2. It does not apply to deliberate release of GMMs" (the definition of "contained use" is given in Box 4 below). This procedure was probably envisaged for organisms used in laboratories and industrial installations, and it is not clear how it might apply to the use of a GMMO in applications that need to be undertaken outside of laboratory containment. It is therefore possible that even if the exclusion was obtained, Prof. Ajioka's team would still need to demonstrate why the use of their device in the field (e.g. in a village in Nepal) would not constitute deliberate release.

The team is however convinced that their GMMO is inherently safe and that deploying it in the way they envisage should not be considered deliberate release. If their approach is successful, it could open up the way for other whole cell living biosensors that use strains of GMMOs that fulfill the criteria set out in Annex II, Part B of Directive 2009/41/EC.

Glass vial containing the ARSOlux biosensor

Portable luminometer used to quantify arsenic concentration in samples

ARSOlux field tests in Vietnam, 2005

ARSOlux field tests in Bangladesh, 2010

Lab-in-a-van used for ARSOlux field test in Saxony, 2011

Source: ARSOlux website:
http://www.ufz.de/arsolux/index.php?en=20675

Photo Credits: ARSOlux

4. "Contained use" and "deliberate release": blurred boundaries

"Contained use" and "deliberate release into the environment" are key categories in the EU/UK regulatory framework (see Figure 1). EU legislation defines "contained use" on the basis of whether or not "specific containment measures" are used "to limit the contact [of the GMO] with and to provide a high level of safety for, the general population and the environment" (Directives 2001/18/EC and 2009/41/EC, see Box 4 below). UK regulations further define such containment measures as "physical, chemical or biological barriers" (UK Genetically Modified Organism (Contained Use) Regulation 2000). In practice, as we saw in the last section, the criteria considered and the measures imposed by regulatory authorities always include social and institutional factors that are not well captured by the terms "physical, chemical or biological".

Box 4: UK and EU Regulatory definitions of "contained use" and "deliberate release"

Definition of "contained use":

In UK Genetically Modified Organisms (Contained Use) Regulations 2000 (Article 2(1)):

"Contained use" means an activity in which organisms are genetically modified or in which genetically modified organisms are cultured, stored, transported, destroyed, disposed of or used in any other way and for which physical, chemical or biological barriers, or any combination of such barriers, are used to limit their contact with, and to provide a high level of protection for, humans and the environment.

In EU Directive 2009/41/EC on the contained use of genetically modified micro-organisms (Article 2(c)):

"Contained use" means any activity in which micro-organisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment.

Definition of "deliberate release"

In EU Directive 2001/18/EC (Article 2.3):

"deliberate release" means any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment".

In the UK Genetically Modified Organisms (Deliberate Release) Regulations 2002:

There is no definition of "deliberate release" in this Regulation, and since it implements EU Directive 2001/18/EC the definition above applies.

Definition of containment in the USA:

In the USA, the Environmental Protection Agency (EPA) uses a similar concept for containment. For R&D to be exempt from reporting, two sets of options pertain:

1) compliance with NIH guidelines; or
2) research and development activities solely within a contained structure supervised by a technically qualified individual with record keeping to document compliance. (Structure was intentionally loosely defined.)

The regulatory definition of containment is open to interpretation and adaptation to circumstances; and this was recognised as early as 1989 by the UK's Royal Commission on Environmental Protection (RCEP, 1989, p. 8):

> *[The definition of containment by the HSE] recognises that no system can ensure complete containment and enables a degree of judgment to be used on what constitutes a release. For example, a genetically engineered sheep in a field might not be considered as released, if the field were adequately fenced to contain it, whereas a genetically engineered microorganism in the same field would be considered as released since field fences are not adequate to contain micro-organisms.*

Such judgements necessarily take place within the regulatory process and decisions are aided and informed by the Scientific Advisory Committee on Genetic Modification (SACGM) Compendium of Guidance. In our interviews, regulators and expert members of advisory committees used medicinal GMMOs as the example where the boundary has been most difficult to define, with one regulator indicating that in some cases the decision could easily go either way. When discussing and explaining these judgements, it might be useful to better acknowledge the inter-related technical and social dimensions of "containment". Thus containment should be understood as encompassing both:

a) **Physical, biological, and chemical measures:** examples of physical measures include limiting work to a demarcated area and ensuring that premises are suitable for easy clean up of accidental spills (e.g. no carpets or porous walls); examples of biological measures (consisting essentially of vector-host pairs) are listed in Box 3 above and in Annex II, Part B of Directive 2009/41/EC (see Appendix 4); and chemical measures include easy access to disinfectants to be used in case of spillage. The measures envisaged by synthetic biologists for "built-in biosafety" or "intrinsic containment" are also forms of biological containment (discussed further in section 5); and the sealed vials used for the ARSOlux and Wellcome Trust funded whole cell biosensors could be considered a physical measure.

b) **Social and institutional measures:** the premises and operators that are awarded permits to conduct GM work are part of recognised institutions, with biological safety committees, team leaders who are responsible for the safety and conduct of those under their supervision, training procedures for staff, measures for internal and external inspection, sanctions for those who breach regulations, and public scrutiny through registries of licensed facilities.

This demonstrates that it is not only the intrinsic properties of the recipient organism, the genetic material transferred, and the vector used in the process that determines whether or not a product made from or containing a GMMOs is defined by regulators as "contained use"; or that will determine the extent to which the GM application is considered to be safe. The conditions under which the GMMO will be used and the characteristics of the user are also crucially important. The GMMO is expected to be used by a person who is adequately trained, experienced, and supervised: thus, the same operation undertaken by a PhD student in a laboratory at a UK university or by a person who is not a professional scientist in a village in the Himalayas or rural Africa will not be assessed in the same way.

Another illustration of this point is provided by a recent application submitted to UK regulatory authorities[14] for a clinical trial involving a GMMO that would be carried out across several different hospitals in the UK that did not have premises already licensed for work with GMMOs, and where the clinicians involved had no prior specialised experience or training in handling GMMOs. In this case, due attention was paid to training needs for the staff involved and to setting up temporary premises within the hospitals, that complied with the needs of Good Laboratory Practice and waste disposal for operations using GMMOs.

These social and institutional dimensions of containment tend to be under-recognised by laboratory researchers, and this helps to explain their enthusiasm for and faith in engineered biological containment measures. Legislative requirements, especially for Class 1 operations, have become an integral part of daily routines in university laboratories and tend to be seen as much as good laboratory practices that ensure the quality of experiments as safety measures to reduce environmental and health risks. As a result, laboratory researchers can be surprised to find out that breaching these practices would be a criminal act under UK and EU legislation, and that team leaders are responsible for ensuring compliance of those they supervise.

[14] Notification B/GB/13/R46/01 by Celladon Corporation for a project entitled "A Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure and Investigation of the safety and feasibility of AAV1/SERCA2a gene transfer in patients with chronic heart failure and a left ventricular assist device", http://gmoinfo.jrc.ec.europa.eu/gmo_report.aspx?CurNot=B/GB/13/R46/01/S.

These social and institutional dimensions do not only affect the definition of "contained use" and "deliberate release" categories, they are also important dimensions for the risk assessment itself, as recognised in the annexes of Directive 2001/18/EC that outline the principles and procedures to be used for the environmental risk assessment of released GMOs:

- Annex II of Directive 2001/18/EC states that the magnitude of the consequence of each adverse effect "is likely to be influenced by the environment into which the GMO(s) are intended to be released and *the manner of the release*" (emphasis added).
- Annex IV of Directive 2001/18/EC requests "additional information" for placing on the market including a "description of how the product and the GMO as or in [a] product are intended to be used", and the "*intended categories of users* of the products, e.g. industry, agriculture, and skilled trades, consumer use by public at large"[15].

The attention given to "DIY biologists" in discussions about the regulation of synthetic biology also reveals the importance of the institutional dimensions of containment. These discussions can give the impression that these amateurs are not subject to the same risk regulations as university researchers or industry operators, but this is not the case: anybody operating in the EU/UK is subject to the same laws. The concern emerges from the fact that these actors are, by definition, not part of recognised professional bodies and recognised institutions, and therefore that their compliance to these laws may be difficult to monitor. However, in practice, the overlap between DIY biologists and university researchers is significant: far from being outsiders, amateur biologists are becoming increasingly professionalised and are proactive in addressing biosafety concerns. All "community labs" that operate publicly foster a culture of responsibility and pledge not to use GMOs, or do so only in facilities that have obtained regulatory approval, often in collaboration with academic university laboratories. An example of this was the collaboration between London BioHackspace and the 2012 UCL iGEM team to develop a public biobrick: the GM work took place in a UCL laboratory to comply with EU/UK regulations (Jefferson, 2013). Paradoxically, and partly perhaps because they have been subjected to such intense media and policy attention (including from an FBI outreach programme), DIY Biologists seem more aware of how their own actions may impact the safe and responsible use GMOs and synthetic biology than university laboratory researchers who unquestioningly abide by regulations.

How contained is contained?

Measures imposed by regulators for contained use do not guarantee one hundred per cent containment. It is often stated that existing containment measures established after the Asilomar conference on recombinant DNA technologies in 1975 have proved their effectiveness as biosafety measures, because there have been no serious health disasters, even though GMMOs have been routinely used in numerous laboratories and factories around the world for several decades. But it is important to recognise that the *absence of evidence of harm is not the same as the evidence of absence of harm*. Rather than serving as evidence of safety, the lack of evidence of harm can be interpreted as evidence that failures in biosafety measures are not sufficiently tracked and investigated. Indeed studies indicate that data on the containment effectiveness of equipment and laboratories are scarce and fragmented (Kimman et al., 2008); and NGOs claim that suspicious illnesses and deaths among staff working with GMMOs in contained use facilities have occurred, and that the lack of evidence in this area is due to the fact that such cases are insufficiently investigated and reported. The case of former Pfizer scientist turned whistle-blower Becky McLain, who was fired after making claims that she had become ill as a result of her laboratory work with GMOs, is emblematic for these NGOs[16].

Furthermore, NGOs have argued that GMMOs are "leaking out" of contained use facilities, with potential health and environmental impacts, because physical containment measures are insufficiently

[15] Annex IV of Directive 2001/18/EC mentions guidance notes to be produced as regards to "the description of how the product is intended to be used" but as far as we have been able to ascertain these have not yet been produced.

[16] For further information about this case, see http://www.nytimes.com/2010/04/03/business/03pfizer.html?_r=0

comprehensive (especially regarding waste streams), routinely flouted, and that infringement of the regulations is not subject to stringent enough penalties or adequate public scrutiny (GeneWatch UK, 1999). Synthetic biologists freely acknowledge that containment in laboratories and industrial facilities cannot be foolproof, and use this as an argument to promote the use of additional engineered biocontainment measures (e.g. Wright et al., 2013, p. 5); but as discussed in the next section these approaches are also proposed as biosafety measures for cases where GMMOs actually need to be released into the environment in order to perform their function.

5. Built-in biocontainment for biosafety

Synthetic biologists are very enthusiastic about the potential for "built-in" or "intrinsic" biocontainment measures, or "genetic safeguards", that could "operate within synthetic organisms themselves to prevent escaped microbes from proliferating unchecked and to prevent the spread of engineered genetic material into unintended host cells" (Moe-Behrens et al., 2013, p. 2) (see also Garfinkel, 2012; Schmidt and de Lorenzo, 2012; Wright et al., 2013). As a result, policy discussions about synthetic biology and environmental risks often revolve around this concept of "building containment mechanisms into synthetic life" (Moe-Behrens et al., 2013). For example, the US Presidential Commission for the Study of Bioethical Issues (2010, p. 68) has endorsed this approach and recommended the use of multiple biosafety mechanisms "to ensure system redundancy in case of component inactivation":

> *internal mechanisms to reliably contain function and reduce or eliminate these risks are being developed. "Biological isolation," which is also termed "biosafety engineering," aims to build in molecular "brakes" or "seatbelts" that restrain growth or replication of partially or fully synthetic organisms. Synthetic organisms can be engineered to be contained physically or temporally. Additional data are needed to assess how well biologically engineered safeguards, such as "kill switches" that activate after a defined number of generations, will work.*

Discussions about environmental risk initiated by synthetic biologists have focused almost exclusively on these approaches; as demonstrated by the series of workshops organised in the USA by SynBERC and the Woodrow Wilson Center[17], and a workshop in Germany funded by the European Science Foundation (Garfinkel, 2012). Governmental research funding agencies in the UK also appear keen to support this approach, as demonstrated by the fact that our workshop was conducted under the auspices of the project "Engineered security systems for environmental synthetic biology", which seeks to design such "security devices", and is co-funded by four UK agencies [18].

These proposals raise four important issues:

(i) Built-in biocontainment cannot fully *prevent* HGT

Synthetic biologists who promote these approaches speak and write about using engineered designs to *prevent* proliferation and/or transfer of genetic material, and this gets understood by policy makers (at least those who are somewhat removed from risk assessment arenas) as "internal mechanisms to *reliably* contain function and reduce or *eliminate* these risks" (quote from Presidential Commission cited above, emphasis added). Yet when questioned, these synthetic biologists will readily acknowledge that no such mechanisms could ever be infallible, because living organisms have well-know mechanism that enable them to evolve to circumvent biosafety devices. This becomes particularly relevant when one considers the scale of releases of GMMOs: if extremely large numbers of cells of GMMOs are released over very large areas and extensive time scales (for example for long-

---

[17] For workshop reports see: "Beyond Containment: Assessing, Testing and Demonstrating Safety on Release of SynBio Devices and Chassis" January 13, 2011, http://www.synbioproject.org/events/archive/6635/; and "Comprehensive Environmental Assessment and Its Application to Synthetic Biology Applications" July 28, 2011, http://www.synbioproject.org/events/archive/cea/

[18] The project is led by Dr Tom Ellis at Imperial College London and funded by the Joint Synthetic Biology Initiative (JSBI), co-funded by the Defence Science and Technology Laboratory (DSTL), Biology and Biotechnology and Biology Sciences Research Council (BBRSC), the Engineering and Physical Sciences Research Council (EPSRC), and Medical Research Council (MRC). http://www.bbsrc.ac.uk/pa/grants/AwardDetails.aspx?FundingReference=BB%2fJ019720%2f1.

term biomonitoring or bioremediation), even an extremely low probability for the failure of the design can lead to a high probability for the proliferation of the organism and/or transfer of genetic material to other organisms (because the probability of proliferation or horizontal gene transfer will be a function of the frequency of the failure of the device, the number of organisms released, the number of releases, the geographical scope of the release and the life-span of the GMMO population). As a result, regulators are not particularly enthusiastic about the prospects for these tools to mitigate potential risks (Pauwels et al., 2013),

(ii) Preventing natural transformation is particularly challenging

The proponents of such solutions argue that it is possible to engineer solutions to prevent horizontal gene transfer that occurs via active mechanisms (e.g. transduction, meaning active transfer via bacteriophages; and conjugation, meaning active transfer via pili), but admit that minimising the incorporation of so-called "free" or "naked" DNA via natural transformation is more of a challenge (Wright et al., 2013). Although the frequency of such transformation in the environment is not well characterised, this process is important for the persistence and spread of DNA in the environment (de Lorenzo and Wackernagel, 1994).

(iii) 'Intrinsic' or 'internal' biocontainment designs fails to address important 'external' factors

Synthetic biologists who promote "intrinsic" engineered designs for biosafety tend to focus their attention on the internal genetic mechanisms of the organisms to be released, but many factors beyond the bacteria's genetics, and outside of the bacterial cell, will also influence the frequency and impact of HGT. Thus, regulators expect applicants to consider a wide range of factors that operate beyond the organism itself. For example European Decision 2002/623/EC provides a list of factors that may affect the speed and extent of gene transfer to other species, which includes: "the reproductive properties of the GMO itself, including the modified sequences, *the conditions of release, and particular environmental considerations such as climate (for example wind), agricultural practices, the availability of hosts for parasites"* (emphasis added); and Annex II of EC Directive 2001/18 stresses that "[t]he magnitude of the consequences is likely to be influenced *by the environment into which the GMO(s) is (are) intended to be released and the manner of the release*" (emphasis added)

(iv) The focus on biocontainment can be seen to imply that HGT is a hazard in itself

The almost exclusive focus on intrinsic biological containment in discussions among synthetic biologists can be interpreted as assuming that the horizontal transfer of genetic material is, in itself, the important adverse effect that needs to be prevented. Interestingly, synthetic biologists share this view with environmental NGOs who are critical of genetic engineering and of synthetic biology, and both groups use terms such as "genetic pollution" and "genetic contamination" to describe this phenomenon. But the perspective of regulators and scientific experts involved in environmental risk assessment is rather different: in these circles, gene transfer is not understood as a hazard or adverse effect in itself; what matters is the identification of adverse effects that might occur as a result of the transfer of genetic material.

Thus, in the European Commission Decision 2002/623/EC that establishes guidance notes for the environmental risk assessment to be conducted prior to deliberate release[19], "the transfer of the inserted genetic material to other organisms, or the same organism, whether genetically modified or not" is listed as one of the *mechanisms* through which an adverse effect could occur, not an adverse effect in itself. European Decision 2002/623/EC also emphasizes that:

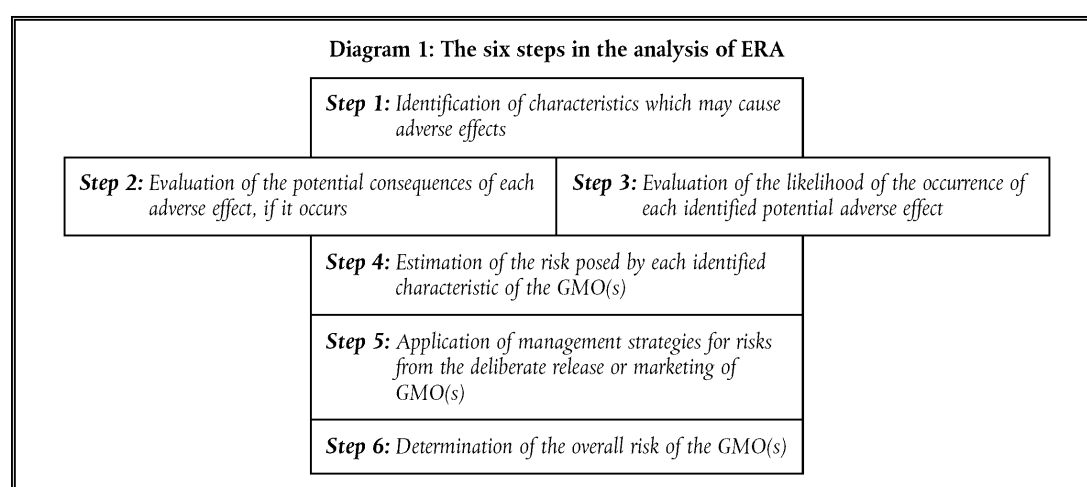
> *[i]n the case of microorganisms, horizontal gene transfer plays a more important role. Certain genetic material can be easily transferred between more closely related organisms, for example, via plasmids or phages. The potential rapid growth rate of microorganisms can enable gene transfer at relatively high levels compared to higher organisms.*

---

[19] 2002/623/EC: Decision of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms.

Annex II of European Directive 2001/18, together with Decision 2002/623/EC, sets out the principles for the environmental risk assessment to be performed prior to deliberate release. The overall schema, summarised in Figure 3, follows the traditional framework for environmental risk assessment, involving the sequential steps of hazard identification, risk evaluation, risk estimation and risk management. An important feature of this kind of environmental risk assessment, which is often overlooked by scientists who are not familiar with these procedures, is that in step 2 of Figure 3, the evaluation of the magnitude of the consequences "*should assume that such an adverse effect will occur*" (emphasis added). Thus, establishing that horizontal gene transfer would be minimised through genetic engineering biocontainment designs would not be sufficient for an environmental risk assessment: it would also be necessary to assess what might happen if and when horizontal gene transfer did occur.

Figure 3: The six steps in Environmental Risk Assessment
(Source: European Decision 2002/623/EC)



6. Broader perspectives on risk and uncertainty

Appraisals of possible future environmental impacts (positive and negative) of a new technology are necessarily made in situations where knowledge is incomplete, and where ambiguity and ignorance prevail. The UK Synthetic Biology Roadmap recognised that one of the key challenges for responsible research and innovation in the field of synthetic biology is the need to take into account such "inescapable uncertainty" (UK Synthetic Biology Roadmap Coordination Group, 2012, p. 19). According to Stirling (2010), where knowledge is incomplete, risk assessment based purely on science-based advice becomes increasingly problematic, and this points to the need for what he has termed "plural and conditional expert advice" which recognises the need to go beyond the question of risk and look at what counts as expertise. From this perspective, a broad range of scientific expertise – from molecular biology to microbial ecology and beyond – as well as expertise from lay people and relevant social groups, needs to be involved in the appraisal process.

Of course it is not possible to reliably foresee the unpredictable, but there are lessons to be learnt from past mistakes. In 2001 the European Environment Agency published retrospective analyses of 14 cases that had become well-known sources of harm, to explore how societies had used, or not, the scientific information available at the time in order to avoid or reduce harmful effects. These included polychlorinated biphenyls (PCBs), the pharmaceutical diethylstilboestrol (DES), asbestos, medical X-rays and mad cow disease. Twelve "late lessons from early warnings" were identified for how to make better decisions in the face of uncertainty (European Environment Agency, 2001):

1. *Acknowledge and respond to ignorance, as well as uncertainty and risk, in technology appraisal and public policymaking*
2. *Provide adequate long-term environmental and health monitoring and research into early warnings*
3. *Identify and work to reduce 'blind spots' and gaps in scientific knowledge*
4. *Identify and reduce interdisciplinary obstacles to learning*
5. *Ensure that real world conditions are adequately accounted for in regulatory appraisal*
6. *Systematically scrutinise the claimed justifications and benefits alongside the potential risks*
7. *Evaluate a range of alternative options for meeting needs alongside the option under appraisal, and promote more robust, diverse and adaptable technologies so as to minimise the costs of surprises and maximise the benefits of innovation*
8. *Ensure use of lay and local knowledge, as well as relevant specialist expertise in the appraisal*
9. *Take full account of the assumptions and values of different social groups*
10. *Maintain the regulatory independence of interested parties while retaining an inclusive approach to information and opinion gathering*
11. *Identify and reduce institutional obstacles to learning and action*
12. *Avoid 'paralysis by analysis' by acting to reduce potential harm when there are reasonable grounds for concern*

For Stirling (2008 and 2010) the key question is how to move away from a narrow focus on risk to broader and deeper understandings of incomplete knowledge; and this would mean asking experts to provide "plural conditional" advice rather that "single definitive" advice. Risk and uncertainty are very different, but when experts providing advice to policy makers acknowledge uncertainty, they tend to do so in ways that reduce unknowns to measurable "risk".

7. Conclusion

This report set down the foundations for the discussions held at the Workshop on "Synthetic biology: containment and release of engineered micro-organisms" that took place at King's College London on 29th April 2013, and will bring together synthetic biologists, microbial ecologists, environmental NGOs, regulators, social scientists, policy analysts, and research funders. Some of the questions to be discussed that emerged from this preliminary research are:

- 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?
- 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?
- What engineering approaches to biocontainment have been proposed by synthetic biologists and to what extent do they provide solutions to biosafety concerns?
- What are the limitations of current governance frameworks for dealing with risk and uncertainty and how could they be addressed?

Appendix 1: Definitions of GMOs in UK legislation

Article 2(1) of the UK Genetically Modified Organisms (Contained Use) Regulations 2000
"Genetic modification" in relation to an organism means the altering of the genetic material in that organism in a way that does not occur naturally by mating or natural recombination or both and within the terms of this definition—

(a) genetic modification occurs at least through the use of the techniques listed in Part I of Schedule 2; and

(b) the techniques listed in Part II of Schedule 2 are not considered to result in genetic Modification.

"Micro-organism" means a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, and includes a virus, a viroid, and an animal or plant cell in culture.

Schedule 2, Part I - Examples of techniques constituting genetic modifications
Examples of the techniques which constitute genetic modification which are referred to in subparagraph (a) of the definition of "genetic modification" in regulation 2(1) are—

(a) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules, produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;

(b) techniques involving the direct introduction into an organism of heritable genetic material prepared outside the organism, including micro-injection, macro-injection and microencapsulation;

(c) cell fusion or hybridization techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Schedule 2, Part II - Techniques which are not considered to result in genetic modification
The following techniques are not considered to result in genetic modification provided that they do not involve the use of genetically modified organisms made by techniques other than those listed in Part III or the use of recombinant nucleic acid molecules, namely—

(a) in vitro fertilisation;

(b) natural processes including conjugation, transduction or transformation;

(c) polyploidy induction.

Appendix 2: Definition of GMOs in EU legislation

Article 2(b) of EU Directive 2009/41/EC on the contained use of genetically modified micro-organisms
'Genetically modified micro-organism' (GMM) means a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination; within the terms of this definition:

(i) genetic modification occurs at least through the use of the techniques listed in Annex I, Part A;

(ii) the techniques listed in Annex I, Part B, are not considered to result in genetic modification;

Annex 1 - Part A
Techniques of genetic modification referred to in point (b)(i) of Article 2 are, inter alia:

1. Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.
2. Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism, including micro-injection, macro-injection and micro-encapsulation.
3. Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Annex 1 - Part B
Techniques referred to in point (b)(ii) of Article 2 which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant-nucleic acid molecules or GMMs made by techniques/methods other than the techniques/methods excluded by Part A of Annex II:

1. in vitro fertilisation;
2. natural processes such as: conjugation, transduction, transformation;
3. polyploidy induction

Article 2(2) of EU Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms
"Genetically modified organism (GMO)" means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;
Within the terms of this definition:
(a) genetic modification occurs at least through the use of the techniques listed in Annex I A, part 1;
(b) the techniques listed in Annex I A, part 2, are not considered to result in genetic modification;

Annex I A - Part 1
Techniques of genetic modification referred to in Article 2(2)(a) are *inter alia*:
(1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;
(2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;
(3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Annex I A - Part 2
Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B:
(1) in vitro fertilisation,
(2) natural processes such as: conjugation, transduction, transformation,
(3) polyploidy induction.

Appendix 3: Definition of Intergeneric Microorganism in US Coordinated Framework for Regulation of Biotechnology

"Those organisms deliberately formed to contain an intergeneric combination of genetic material; excluded are organisms that have resulted from the addition of intergeneric materials that is well-characterized and contains only non-coding regulatory regions such as operators, promoters, origins of replication, terminators and ribosome binding regions.

"Well-characterized and contains only non-coding regulatory regions" means that the producer of the microorganism can document the following:
a. The exact nucleotide base sequence of the regulatory region and any inserted flanking nucleotides;
b. The regulatory region and any inserted flanking nucleotides do not code independently for a protein, peptide of functional RNA molecules;
c. The regulatory region solely controls the activity of other sequences that code for protein or peptide molecules or act as recognition sites for the initiation of nucleic acid or protein synthesis."

Source: http://www.epa.gov/biotech_rule/pubs/pdf/coordinated-framework-1986.pdf

US EPA definition of Intergeneric Microorganisms

"EPA believes that intergeneric microorganisms have a sufficiently high likelihood of expressing new traits or new combinations of traits to be termed "new" and warrant review. Microorganisms that are not intergeneric are not considered "new", and thus are not subject to reporting under section 5 of TSCA [the Toxic Substances Control Act]. When defining "intergeneric microorganism," in the case of chemically synthesized genes, the Agency has followed a similar principle. The genetic sequence of the synthesized gene may be identical to a sequence known to occur in an organism in the same genus as the recipient microorganism. If so, the resulting microorganism is considered intrageneric and thus not new. Conversely, the sequence of the synthesized gene may be different or not known to be identical to a sequence in the genus of the recipient microorganism, in which case, the resulting product is considered intergeneric."
Source: http://www.epa.gov/biotech_rule/pubs/fs-001.htm

Appendix 4: Procedure to exclude a GMMO from the scope of Directive 2009/41/EC on contained use

The introduction to Part B of Annex II of Directive 2009/41/EC states that:

> *Types of GMMs listed in Part C in accordance with the regulatory procedure with scrutiny referred to in Article 20(2) are excluded from the scope of this Directive. GMMs will be added to the list on a case-by-case basis and exclusion will relate only to each clearly identified GMM. This exclusion applies only when the GMM is used under conditions of contained use as defined in point (c) of Article 2. It does not apply to the deliberate release of GMMs. For a GMM to be listed in Part C, it must be proved that it meets the criteria given below.*

Article 20(2) of Directive 2009/41/EC states that:

> *Where reference is made to this paragraph, Article 5a(1) to (4) and Article 7 of Decision 1999/468/EC shall apply, having regard to the provisions of Article 8 thereof.*

Decision 1999/468/EC lays down "the procedures for the exercise of implementing powers conferred on the Commission"; and Article 5 (1) to (4) and Article 7 of Decision 1999/468/EC set out the regulatory procedure for the use of the "regulatory committee" that delivers opinions on draft measures prior to Member States voting on whether or not to adopt draft measures.

Criteria establishing the safety of GMMs for human health and the environment (Directive 2009/41/EC, Annex II, Part B) (GMMs that do not meet these criteria may not be included in Part C)

2.1. Strain verification/authentication

Identity of the strain must be precisely established. Modification must be known and verified.

2.2. Documented and established evidence of safety

Documented evidence of the safety of the organism must be provided.

2.3. Genetic stability

Where any instability could adversely affect safety, evidence of stability is required.

3. **Specific criteria**

3.1. Non-pathogenic

The GMM should not be capable of causing disease or harm to a healthy human, plant or animal. Since pathogenicity includes both toxigenicity and allergenicity, the GMM should therefore be:

3.1.1. Non-toxigenic

The GMM should not produce increased toxigenicity as a result of the genetic modification nor be noted for its toxigenic properties.

3.1.2. Non-allergenic

The GMM should not produce increased allergenicity as a result of the genetic modification nor be a noted allergen, having, for example, allergenicity comparable in particular with that of the micro-organisms identified in Directive 2000/54/EC.

3.2. No harmful adventitious agents

The GMM should not harbour known harmful adventitious agents such as other micro-organisms, active or latent, existing alongside or inside the GMM, that could cause harm to human health and the environment.

3.3. Transfer of genetic material

The modified genetic material must not give rise to harm if transferred; nor should it be self-transmissible or transferable at a frequency greater than other genes of the recipient or parental micro-organism.

3.4. Safety for the environment in the event of a significant and unintended release

GMMs must not produce adverse effects on the environment, immediate or delayed, should any incident involving a significant and unintended release occur.

References

All websites cited were last accessed on 23/07/2013.

Bailey, C., Metcalf, H., & Crook, B. (2012). *Synthetic Biology: a review of the technology, and current and future needs from the regulatory framework in Britain*. Report prepared by the Health and Safety Laboratory for the Health and Safety Executive.

Bailey, P. (2013). "Feature: The biggest poisoning in history." *Wellcome Trust News and Features*. http://www.wellcome.ac.uk/News/2013/Features/WTP051176.htm

BBSRC (2008). *Synthetic Biology Regulators' Meeting: Public statement, 3 October 2008*. http://www.bbsrc.ac.uk/web/FILES/Policies/synthetic_biology_public_statements.pdf

Bosworth, A. H., M. K. Williams, K. A. Albrecht, R. Kwiatkowski, J. Beynon, T. R. Hankinson, C. W. Ronson, F. Cannon, T. J. Wacek and E. W. Triplett (1994). "Alfalfa yield response to inoculation with recombinant strains of Rhizobium meliloti with an extra copy of dctABD and/or modified nifA expression." *Appl. Environ. Microbiol.*, 60: 3815-3832.

Bull, A. T., G. Holt and M. D. Lilly (1982). *Biotechnology: International Trends and Perspectives defines biotechnology*. Paris: OECD.

de Lorenzo, V. (2010). "Environmental biosafety in the age of synthetic biology: do we really need a radical new approach?" *BioEssays*, 32(11): 926-931.

de Lorenzo, M., G. and W. Wackernagel (1994). "Bacterial gene transfer by natural genetic transformation in the environment." *Microbiological Reviews*, 58(3): 563-602.

European Environment Agency (2001). *Late lessons from early warnings: the precautionary principle 1986-2000. Environmental Issues Report*. Copenhagen: EEA.

French, C. E., K. de Mora, N. Joshi, A. Elfick, J. Haseloff and J. Ajioka (2011). "Synthetic Biology and the Art of Biosensor Design". In Institute of Medicine (US) Forum on Microbial Threats, *The Science and Applications of Synthetic and Systems Biology: Workshop Summary*. Washington DC: National Academies Press. http://www.ncbi.nlm.nih.gov/books/NBK84465/

Friends of the Earth, ICTA and ETC Group (2012). *The Principles for the Oversight of Synthetic Biology*. http://www.foe.org/news/archives/2012-03-global-coalition-calls-oversight-synthetic-biology

Garfinkel, M. (2012). *ESF/LESC Strategic Workshop on Biological containment of synthetic microorganisms: science and policy. Heidelberg (Germany), 13-14 November 2012. Scientific Report* http://www.embo.org/documents/science_policy/biocontainment_ESF_EMBO_2012_workshop_report.pdf

GeneWatch UK (1999). Leaking From The Lab? The 'Contained' Use of Genetically Modified Micro-organisms in the UK. http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/Contained_Use_Report___Final_A4.doc

HSE (2007). *The SACGM Compendium of Guidance*. London: Health and Safety Executive. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/

Hernández, M., M. Pla, T. Esteve, S. Prat, P. Puigdomènech and A. Ferrando (2003). "A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3'-transgene integration sequence." *Transgenic Research*, 12: 179-189.

Hirsch, P. R. (2004). "Release of transgenic bacerial inoculants - rhizobia as a case study." *Plant and Soil*, 266: 1-10.

Huang, D. (2002). "Research and development of recombinant microbial agents and biosafety consideration in China". In *The 7th International Symposium on the Biosafety of Genetically Modified Organisms, Beijing, China, 10-16 October 2002*, pp 117-120. http://www.isbr.info/sites/default/files/editors/symposia/7th_symposium-2002.pdf

Jefferson, C. (2013). *Governing Amateur Biology: Extending Responsible Research and Innovation in Synthetic Biology to New Actors*. Research report for the Wellcome Trust Project on "Building a Sustainable Capacity in Dual-Use Bioethics". http://www.brad.ac.uk/bioethics/media/ssis/bioethics/docs/Jefferson_Governing_Amateur_Biology.pdf

Kimman, T. G., E. Smit and M. l. R. Klein (2008). "Evidence-Based Biosafety: a Review of the Principles and Effectiveness of Microbiological Containment Measures." *Clinical Microbiology Reviews*, 21(3): 403-425.

Kitney, R., and Freemont, P. (2012). Synthetic Biology - the state of play. *FEBS Letters*, 586: 2029-2036.

McDougall, A. (2011). First sale of Amyris' renewable squalane. Cosmetics design.com USA. http://www.cosmeticsdesign.com/Business-Financial/First-sale-of-Amyris-renewable-squalane

Moe-Behrens, G. H. G., R. Davis and K. A. Haynes (2013). "Preparing synthetic biology for the world." *Frontiers in Microbiology*, 4: 1-10.

O'Flaherty, S., Y. Moënne-Loccoz, B. Boesten, P. Higgins, D. N. Dowling, S. Condon and F. O'Gara (1995). "Greenhouse and field evaluations of an autoselective system based on an essential thymidylate synthase gene for improved maintenance of plasmid vectors in modified Rhizobium-meliloti." *Appl. Environ. Microbiol.* 61: 4051-4056.

OECD (1986). *Recombinant DNA Safety Considerations*. Paris, OECD.

Pauwels, K., Mampuys, R., Golstein, C., Breyer, D., Herman, P., Kaspari, M., . . . Schönig, B. (2013). Event report: SynBio Workshop (Paris 2012) - Risk assessment challenges of Synthetic Biology. *Journal of Consumer Protection and Food Safety*, published online 03/07/2013. doi: 10.1007/s00003-013-0829-9

Podevin, N. and P. du Jardin (2012). "Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants." *GM Crops Food*, 3(4): 296-300.

Rang, A., B. Linke and B. Jansen (2005). "Detection of RNA variants transcribed from the transgene in Roundup Ready soybean." *European Food Research Technology*, 220: 438-443.

Rodemeyer, M. (2009). *New Life, Old Bottles*. Washinton DC: Woodrow Wilson International Center for Scholars. http://www.synbioproject.org/library/publications/archive/synbio2/

Ronson, C. W., A. Bosworth, M. Genova, S. Gudbrandsen, T. Hankinson, R. Kwiatkowski, H. Ratcliffe, C. Robie, P. Sweeney and W. Szeto (1990). "Field release of genetically-engineered Rhizobium meliloti and Bradyrhizobium japonicum strains". In P. M. Gresshoff, L. E. Roth, G. Stacey and W. E. *Newton (Eds) Nitrogen Fixation: Achievements and Objectives. Proccedings of the 8th International Congress on Nitrogen Fixation, Knoxville, Tennesse, USA, May 20-26 1990*. London: Chapman and Hall. pp 397-404.

RCEP (1989). *The Release of Genetically Engineered Organisms to the Environment. Thirteenth Report. Royal Commission on Environmental Protection*. London: HMSO.

Sanofi (2013). "Sanofi and PATH announce the Launch of Large-scale Production of Semisynthetic Artemisinin against Malaria". Press Release 11/04/2013. http://www.path.org/news/pr130411-ssart-launch.php

Schmidt, M. and V. c. de Lorenzo (2012). "Synthetic constructs in/for the environment: Managing the interplay between natural and engineered Biology." *FEBS Letters*, 586(15): 2199-2206.

Selbitschka, W., D. Jording, S. Nieman, R. Schmidt, A. Puhler, T. Mendum and P. Hirsch (1995). "Construction and characterization of a Rhizobium leguminosarum biovar viciae strain designed to assess horizontal gene transfer in the environment." *FEMS Microbiology Letters*, 128: 255-263.

Selbitschka, W., M. Keller, R. Miethling-Graff, U. Dresing, F. Schwieger, I. Krahn, I. Homann, T. Dammann-Kalinowski, A. Puhler and C. C. Tebbe (2006). "Long-term field release of bioluminescent Sinorhizobium meliloti strains to assess the influence of a recA mutation on the strains' survival." *Microbial Ecology*, 52: 583-595.

Siegfried, K., C. Endes, A. F. M. K. Bhuiyan, A. Kuppardt, J. Mattusch, J. R. van der Meer, A. Chatzinotas and H. Harms (2012). "Field testing of arsenic in groundwater samples of Bangladesh using a test kit based on lyophilized bioreporter bacteria." *Environmental Science & Technology,* 46: 3281-3287.

Snow, A. A., D. A. Andow, P. Gepts, E. M. Hallerman, A. Power, J. M. Tiedje and L. L. Wolfenbarger (2005). "Genetically Engineered Organisms and the Environment: Current Status and Recommendations." *Ecological Applications*, 15(2): 377-404.

Stirling, A. (2008). "Opening up" and "closing down": power, participation and pluralism in the social appraisal of technology." *Science, Technology & Human Values*, 33(2): 262-294.

Stirling, A. (2010). "Keep it Complex." *Nature*, 468: 1029-1031.

Synthetic Biology Project (2012). *Inventory of Synthetic Biology Products - Existing and Possible*. Washinton DC: Woodrow Wilson International Center for Scholars. http://www.synbioproject.org/process/assets/files/6631/_draft/synbio_applications_wwics.pdf

Tiedje, J. M., R. K. Colwell and et al. (1989). "The planned introduction of genetically engineered organisms: ecological considerations and recommendations." *Ecology*, 70(2): 298-315.

Trang, P. T. K., M. Berg, P. H. Viet, N. V. Mui and J. R. van der Meer (2005). "Bacterial bioassay for rapid and accurate analysis of arsenic in highly variable groundwater samples." *Environmental Science and Technology*, 39: 7625-7630.

UK Synthetic Biology Roadmap Coordination Group (2012). *A Synthetic Biology Roadmap for the UK*. London: Technology Strategy Board.

http://webarchive.nationalarchives.gov.uk/20130221185318/www.innovateuk.org/_assets/tsb_syntheticbiologyroadmap.pdf

US Presidential Commission for the Study of Bioethical Issues (2010). *New Directions: the ethics of synthetic biology and emerging technologies*. Washington DC: PCSBI. http://bioethics.gov/synthetic-biology-report

Viebahn, M., E. Smit, D. C. M. Glandorf, K. Wernars and P. A. H. M. Bakker (2009). Effect of gnetically modified bacteria on ecosystems and their potential benefits for bioremediation and biocontrol of plant diseases - a review. In E. Lichtfouse (Ed.) *Climate Change, Intercropping, Pest Control and Benificial Microorganisms, Sustainable Agriculture Reviews Volume 2*. London: Springer. pp 45-69. http://link.springer.com/chapter/10.1007%2F978-90-481-2716-0_4#page-1

Windels, P., I. Taverniers, A. Depicker, E. Van Bockstaele and M. De Loose (2001). "Characterisation of the Roundup Ready soybean insert." *European Food Research Technology*, 213: 107-112.

Wright, O., G.-B. Stan and T. Ellis (2013). "Building-in Biosafety for Synthetic Biology." *Microbiology*, 159: 1221–1235.

Yong, E. (2013). "Can we save the world by re-mixing Life?" *Phenomena, A science salon hosted by National Geographic Magazine*. http://phenomena.nationalgeographic.com/2013/04/11/can-we-save-the-world-by-remixing-life/

Zhang, J., C. Marris and N. Rose (2011). *The International Governance of Synthetic Biology: Scientific uncertainty, cross-borderness and the 'art' of governance*. London: BIOS Centre, London School of Economics. http://royalsociety.org/uploadedFiles/Royal_Society_Content/policy/publications/2011/4294977685.pdf