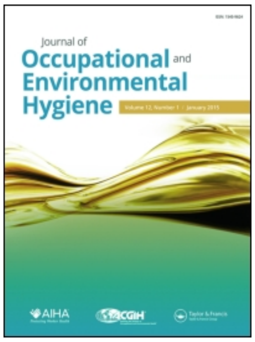

Taylor & Francis
Taylor & Francis Group

Journal of Occupational and Environmental Hygiene

ISSN: 1545-9624 (Print) 1545-9632 (Online) Journal homepage: http://www.tandfonline.com/loi/uoeh20

Synthetic biology and occupational risk

John Howard, Vladimir Murashov & Paul Schulte

To cite this article: John Howard, Vladimir Murashov & Paul Schulte (2017) Synthetic biology and occupational risk, Journal of Occupational and Environmental Hygiene, 14:3, 224-236, DOI: 10.1080/15459624.2016.1237031

To link to this article: http://dx.doi.org/10.1080/15459624.2016.1237031

JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE
2017, VOL. 14, NO. 3, 224–236
http://dx.doi.org/10.1080/15459624.2016.1237031

Taylor & Francis
Taylor & Francis Group

Synthetic biology and occupational risk

John Howard, Vladimir Murashov, and Paul Schulte

National Institute for Occupational Safety and Health, Washington, DC

ABSTRACT

Synthetic biology is an emerging interdisciplinary field of biotechnology that involves applying the principles of engineering and chemical design to biological systems. Biosafety professionals have done an excellent job in addressing research laboratory safety as synthetic biology and gene editing have emerged from the larger field of biotechnology. Despite these efforts, risks posed by synthetic biology are of increasing concern as research procedures scale up to industrial processes in the larger bioeconomy. A greater number and variety of workers will be exposed to commercial synthetic biology risks in the future, including risks to a variety of workers from the use of lentiviral vectors as gene transfer devices. There is a need to review and enhance current protection measures in the field of synthetic biology, whether in experimental laboratories where new advances are being researched, in health care settings where treatments using viral vectors as gene delivery systems are increasingly being used, or in the industrial bioeconomy. Enhanced worker protection measures should include increased injury and illness surveillance of the synthetic biology workforce; proactive risk assessment and management of synthetic biology products; research on the relative effectiveness of extrinsic and intrinsic biocontainment methods; specific safety guidance for synthetic biology industrial processes; determination of appropriate medical mitigation measures for lentiviral vector exposure incidents; and greater awareness and involvement in synthetic biology safety by the general occupational safety and health community as well as by government occupational safety and health research and regulatory agencies.

KEYWORDS
Biocontainment; bioeconomy; gene editing, lentivirus, synthetic biology, viral vectors

Introduction

Synthetic biology is an emerging interdisciplinary field of biotechnology that involves applying the principles of engineering and chemical design to biological systems.[1] The earliest use of the term was by a French biologist in 1910.[2] By 1974, synthetic biology was being viewed as the next phase in molecular biology by which scientists would "devise new control elements and add these new modules to the existing genomes or build up wholly new genomes."[3] In 1978, the Nobel Prize in Physiology or Medicine was awarded to two Americans and one Swiss researcher for their discovery of programmable restriction enzymes (nucleases) which enabled a new era in biotechnology "where not only existing genes are described and analyzed but also new gene arrangements can be constructed and evaluated."[4]

Several definitions of synthetic biology can be found and no widely accepted definition exists. In 2015, the *Ad Hoc Technical Expert Group on Synthetic Biology* of the United Nations Convention on Biological Diversity proposed an operational definition of synthetic biology as "a further development and new dimension of modern biotechnology that combines, science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems."[5]

Although it is often difficult to demarcate synthetic biology from other biotechnology research areas, synthetic biology can be understood to involve two closely related capabilities both of which may have wide utility in commerce and medicine. First, while the transfer of already existing genes from one cell to another characterized an earlier phase of the field of biotechnology, synthetic biology involves the design, assembly, synthesis, or manufacture of *new* genomes, biological pathways, devices, or organisms not found in nature. These operations are made possible by recent advances in DNA synthesis and DNA sequencing, providing standardized DNA "parts," modular protein assemblies, and engineering models.[6-7] Recent achievements in constructing

CONTACT Vladimir Murashov vem8@cdc.gov National Institute for Occupational Safety and Health, 395 E Street, S.W., Washington, DC 20201.

new genomes from DNA sequences include synthesis of a completely new chromosome;[8] a new bacterial genome;[9] the first synthetic life form, a single-celled organism based on an existing bacterium;[10] and living protocells assembled entirely from nonliving, individual biological components.[11-12] Science involving this latter category of "new-to-nature" organisms is a subfield of synthetic biology called *xeno*biology because these organisms are assembled from nonliving chemicals and differ in every biochemical aspect from existing organisms, including exhibiting a very different type of genetic code.[13]

A second capability of synthetic biology involves the re-design of existing genes, cells, or organisms for the purpose of gene therapy.[14] Modification of existing genes in animal and human cells is enabled by a new genome-editing technique known as a clustered regularly interspaced short palindromic (CRISPR)-associated system (Cas].[15] Recent highlights in gene editing include correcting a genetic mutation causing Duchenne muscular dystrophy using a mouse muscle cell model;[16-18] correcting a mutation in mice associated with a human disease called tyrosinemia;[19] and eliminating the AIDS-causing HIV-1 genome from infected human T-lymphoid cells.[20] Progress in this branch of synthetic biology has yielded remarkable therapeutic advances in gene therapy well beyond the achievements of conventional drugs and biologic agents.[21]

The application of engineering principles to fundamental components of biology by combining new sequences of DNA not found in nature has been perceived as the manufacture of new life "from scratch," tantamount to "playing God," and has raised ethical questions whether synthetic biology will alter our concept of what life it.[22-24] One vision for synthetic biology—transhumanism—foresees genetically improving human qualities beyond those controlled by "evolution's blind process of random variation," which has provoked strong ethical and political condemnation.[25] The scientific possibilities of synthetic biology, such as reconstituting the 1918 "Spanish" influenza virus,[26] enhancing the pathogenicity, or transmissibility of existing pathogens ("gain-of-function"),[27] or engineering microbes by "Do-It-Yourself" (DIY) methods,[28] both excite and frighten, often at the same time.[29] An example is a report that scientists have begun privately discussing using a "synthetic genome to create human beings without biological parents."[30]

Societal risks

Synthetic biology promises both tremendous societal benefits in treating human genetic disease,[31] as well as huge commercial market potential for technology investors.[32] At the same time, synthetic biology has raised concerns about potential biosafety and biosecurity risks to researchers, clinicians, and to society in general.

The biosafety concerns about synthetic biology and its gene-editing tools are similar to the concerns lodged about recombinant DNA technology when genetic engineering was first introduced in the early 1980s.[33] However, recent reports of potential exposure incidents to workers at Level 3 and Level 4 biocontainment laboratories only serve to increase biosafety concerns about synthetic biology.[34-35] A strong perception exists that biosafety rules cannot keep up with practices in the modern biotechnology laboratory.[36]

When gene editing was first applied to modifying human embryos,[37] it sparked alarm about making heritable changes to the human germline,[38] and there were calls from prominent scientists for a moratorium on editing the human germline.[39,40] In addition, many civil society groups from various countries have called for a general moratorium on synthetic biology research involving designing, re-designing, and synthesizing plant, animal and human genes until the potential societal risks can be thoroughly studied and controlled.[41,42] The main concern is that despite the benefits of synthetic biology, it is possible that such research can be used for dual—both benevolent and harmful—uses.[43]

As a type of dual use research of concern (DURC), synthetic biology has the potential to post "a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security." [44] The deliberate misuse of synthetic biology for purposes of bioterrorism may be unlikely, but the "de-skilling" of biological research and the emergence of the DIY community raises concerns.[45] A "lone operator" with expertise in synthetic biology, not restricted by institutional biosafety oversight, and intent on doing harm, or a "biohacker" that wants to wreak havoc among living organisms just as his fellow hacker creates viruses that infect and disable computer systems, are distinct possibilities.[46,47]

Risk governance

United States

Since biotechnology arose as a scientific area of research in the 1980s, a range of risk governance strategies have been proposed in the United States and in Europe. In 1986 the earliest U.S. risk governance strategy arose when the White House Office of Science Technology and Policy (OSTP) developed the U.S. government's *Coordinated Framework for the Regulation of Biotechnology* (CF).[48]

As a result of the CF, the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the U.S. Department of Agriculture (USDA), the National Institutes of Health (NIH), and the Occupational Safety and Health Administration (OSHA) outlined their respective roles in ensuring the safety of biotechnology research and products.

In the 1986 CF, OSHA determined that the general duty clause, together with a set of existing occupational safety and health standards, provided an adequate and enforceable basis for protecting biotechnology workers and that no new standards were necessary. OSHA also provided a set of guidelines for biotechnology laboratory worker safety based on existing standards. Specific OSHA standards that may be applicable to biotechnology laboratories include: (1) bloodborne pathogens;[49[(2) toxic and hazardous substances; [50] (3) access to employee exposure and medical records;[51] (4) hazard communication; [52] (5) exposure to toxic chemicals in laboratories; [53] (6) respiratory protection; [54] and (7) safety standards of a general nature, e.g., general environmental, walking and working surfaces, fire protection, compressed gases, electrical safety, and material handling and storage contained in 29 CFR Part 1910 Subparts J, D, E and L, H, S, and N). As a part of the 1986 CF comment process, the National Institute for Occupational Safety and Health (NIOSH) recommended increased injury and illness surveillance of biotechnology workers given the gap in information about risks to such workers.[55]

The 1986 CF was updated in 1992.[56] In 2015, a new process to update the CF was begun by the OSTP. In a White House Memorandum, the EPA, FDA, and USDA was directed to update the CF to clarify agency roles in regulating biotechnology products and to formulate a long-term strategy to ensure that the federal regulatory system is equipped to efficiently assess any risks of future biotechnology products.[57] Listening sessions have been held across the country to receive feedback on illustrative examples for developers who believe they have, or are uncertain as to whether they may have, a biotechnology product that is subject to regulation under one or more of the Federal laws described in the current CF.[58] Additionally, the USDA has asked the U.S. National Academies of Sciences, Engineering and Medicine to evaluate what advances will be made in biotechnology in the next 5–10 years.[55]

The 2015 Memorandum does not explicitly mention risks from occupational exposure to synthetic biology products nor requires OSHA to conduct a regulatory review of synthetic biology. OSHA is, however, considering the need for a standard to ensure that employers establish a comprehensive infection control program and control measures to protect employees from aerosol exposures to infectious agents that can cause significant disease. OSHA has published an *Infectious Diseases Request for Information* (RFI), held stakeholder meetings, conducted site visits, and completed the *Small Business Regulatory Enforcement Fairness Act* (SBREFA) process in support of rulemaking. OSHA plans use information from these sources to further refine its development of a Notice of Proposed Rulemaking for an Infectious Diseases standard that is scheduled for publication in December 2016.[56]

Private sector groups have also called for improvements in the regulatory infrastructure to address the implications of new synthetic biology products.[57-60] Public interest groups have recommended applying the precautionary principle to any further research and commercialization of synthetic products until specific biosafety and biosecurity mechanisms can be developed to keep pace with synthetic biology advances.[61] Other groups have proposed detailed risk governance polices for commercial entities, users, and organizations that engage in synthetic genomics research, including compiling a manual specifically addressing “biosafety in synthetic biology laboratories.”[62]

Biosafety guidance that is specific to scientific advances from synthetic biology would be helpful as both the World Health Organization’s *Laboratory Biosafety Manual* [63] and *Biosafety in Microbiological and Biomedical Laboratories*, [64] jointly co-authored by the U.S. Centers for Disease Control and Prevention and the National Institutes of Health in the U.S. Department of Health and Human Services, address an earlier phase of biotechnology aimed primarily at prevention of exposure to already existing pathogens in traditional biology laboratories. However, synthetic biology is using newly designed organisms and viral vectors (i.e., tools used to deliver genetic material inside a cell), not unmodified existing pathogens. Furthermore, synthetic biology often is not being performed solely by “biologists,” but by engineers, physical scientists, and DIYers not familiar with fundamental biosafety measures such as biocontainment.

Based on recommendations by the National Science Advisory Board for Biosecurity,[68] NIH updated its 2009 Guidelines for Research Involving Recombinant DNA Molecules in 2013 to address the creation and use of organisms and viruses containing synthetic nucleic acid molecules.[69] Under the 2013 NIH Guidelines, investigators must initially assess the risk of the agent to cause disease in laboratory workers or others if a release occurs. After the disease risk is assessed, a decision must be made as to the level of containment to control potential exposure. In determining the level of containment, factors such as virulence, pathogenicity, infectious dose, environmental stability, transmissibility, quantity, availability of treatment, and gene product effects such as toxicity, physiological activity, and allergenicity should be considered.[69]

The 2013 NIH Guidelines make clear that in the case of an organism containing genetic sequences from multiple sources, the potential for causing human disease based on the source(s) of the DNA sequences requires an assessment of the virulence and transmissibility functions encoded by these sequences. Combining sequences in a new biological organism may result in an organism whose risk profile could be higher than that of the contributing organisms or sequences. Based on all these considerations, the appropriate biosafety level (BSL) containment conditions (Levels 1–4) can be selected.[69]

Many of the guidelines aimed at ensuring biosafety for synthetic biology also protect against biosecurity risks. Augmenting guidelines for biosafety, the U.S. government has explicitly addressed dual use research with the intention of raising awareness and limiting the potential for misuse of scientific information derived from life sciences research. In 2015, the *U.S. Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern* set out the guiding principles for the oversight of life sciences dual use research and the responsibilities of research institutions funded with federal funds.[70]

Europe

In Europe, the potential biosafety and biosecurity risks of synthetic biology have also been under review. Government agencies and private sector groups advising European governments have noted the need for robust risk assessments in synthetic biology for the protection of workers and the public. The United Kingdom,[71] Belgium,[72] and the Netherlands[73] have each issued reports about biosafety considerations and regulatory needs in response to the growing field of synthetic biology.

In 2015, the European Commission's (EC) combined scientific committees on health and environmental risks, emerging and newly identified health risks, and consumer safety addressed the question of whether existing methodologies were appropriate for assessing the potential risks associated with synthetic biology research. The Committees' opinion was that existing risk assessment approaches for genetically modified organisms (GMOs) are generally applicable to synthetic biology, but nevertheless noted that "combining genetic parts and the emergence of new properties …will require improving existing methodologies."[74] The EC called for the standardization of risk assessments for synthetic biology, more research to improve the ability to predict the behavior of complex engineered organisms, and doubted that intrinsic biocontainment strategies like intrinsic genetic "safety locks," were effective as a primary strategy to control the risks of synthetic biology.[75] Several other U.S. and European regulations, rules and guidelines addressing risk governance for synthetic biology in agriculture, food and drugs, select agents, and other applications outside of medical applications have been developed.[76] The challenge for both U.S. and European policymakers is to prevent the deliberate or inadvertent use of synthetic biotechnology for harmful purposes without impeding the pathway to the societal benefits this new technology offers.[77]

Much of the impetus for new U.S. and European risk governance strategies as well as concerns about the capabilities of synthetic biology arise from the use of CRISPR, a powerful gene editing technology.[78] CRISPR is what makes genome editing possible and has been hailed as the biggest game changer in biology since development of polymerase chain reaction technology.[79-81] As a disruptive innovation in biology, it is being applied widely. For instance, regulators in the United Kingdom recently approved experiments to switch genes on and off in human embryos using CRISPR genome editing.[82] CRISPR is also being teamed up with gene transfer vehicles or vectors made from viruses. One viral vector being used in synthetic biology—lentiviral vectors—pose some potentially unique risks to workers exposed to them.

Lentiviral vectors and occupational risk

Since the first gene therapy trial in 1990,[83] various types of viruses have been used to create gene transfer devices or "vectors," such as retrovirus, adenovirus, adeno-associated virus and herpes simplex virus.[84,85] Vectors made from members of the *Retroviridae* (retroviruses) family have gained attention as efficient gene transfer vehicles.[86,87] All retroviruses have the ability to transcribe their single stranded RNA genome into double-stranded DNA by the reverse transcriptase enzyme which can then be integrated into the host cell genome, producing permanent genetic change in the organism.[88] Lentiviruses are a subgroup of retroviruses that are found in humans and animals and are characterized by long incubation periods and persistent infection.[89]

The most biologically characterized lentivirus is the human immunodeficiency, type 1 virus (HIV-1). As the causative agent of the acquired immunodeficiency syndrome (AIDS), HIV-1 has been well studied. HIV-1 can spread from person to person through various types of sexual and blood contact. In the occupational setting, HIV-1 transmission can occur through: (1) direct mucosal (splash), or broken skin (cuts), contact with animal and human blood or bodily fluids containing HIV; or (2) percutaneous exposure through parenteral inoculation by means of needlesticks or other types of sharps injuries with blades, glass contaminated with HIV.[90]

HIV-1 envelope proteins recognize a specific subset of lymphocytes called CD4 cells as its primary receptor. CD4 cells are crucial to a well-functioning human immune system. Infection with HIV-1 results in the insertion of viral genes into the nucleus of the CD4 cell where it can remain dormant for years. When reactivated, HIV reproduction results in CD4 cell death. As the population of CD4 cells are depleted, progressive immunodeficiency results leaving the body vulnerable to a wide range of infections that it would have otherwise been able to defend against.[91]

Lentiviral vectors (LVVs) made from HIV-1 are used for gene therapy because the action of its reverse transcriptase and integrase enzymes permits the insertion of the transferred genetic material into the genome of the host, thereby making a permanent genetic change to the target cell.[92] In 2015, LVVs were used in 114 viral vector-based clinical trials.[93]

LVVs derived from HIV-1 are unique members of the retroviral family because they have the ability to infect dividing and non-dividing cells (e.g., nerve, muscle, liver cells) by entering the cell's nucleus, achieving efficient and stable integration, and long-term transgene expression.[94] The ability to infect non-dividing cells greatly expands the scope of LVVs for therapeutic gene transfer applications.

A method that allows an HIV-1-derived LVV to transduce an even wider set of cells than just CD4 cells involves modifying the HIV-1 envelope glycoproteins responsible for cellular attachment through "pseudotyping" with glycoproteins from another virus.[95] Pseudotyping enhances the ability for a LVV to infect specific host cells. Most LVVs in use today are pseudotyped with the glycoprotein G of the vesicular stomatitis virus (VSV-G) which transduces all cell types, greatly increasing the range of cell types such a pseudotyped HIV-1-derived LVV can transduce.[95] However, this increased tropism, or specificity of a LVV for a particular host tissue, can also result in the unintended transduction of "off-target" cell types in a worker who becomes occupationally exposed to VSV-G pseudotyped LVVs.

Pseudotyping LVVs illustrates just one of the risks that synthetic biology researchers, clinicians, and ancillary workers face when they are occupationally exposed to LVVs. The other risks associated with unintentional LVV worker exposure are the potential for generation of replication-competent lentivirus,[96] and for insertional mutagenesis and the transactivation of neighboring genome sequences which could lead to cancer and other diseases.[97]

Replication Competent Lentivirus. The potential for the generation of replication-competent lentivirus (RCL) can occur during viral vector manufacturing, during a research experiment, and during therapeutic use. Lentiviruses have high mutation recombination rates and the risk that HIV could acquire the ability to self-replicate even though a LVV was designed as non-self-replicating is real. This concern has led to at least four generations of refinements in LVV packaging system by separating the transfer vector, the packaging genes and the envelope gene into different plasmids to reduce the risk of replication.[98] The use of new packaging lines have led to the ability of researchers to generate large quantities of LVV that can be used safely for gene delivery in both research and therapeutic applications. Although the risk of recombination to produce fully-competent RCL in a self-inactivating (SIN) vector system is extremely small, it is not zero. Recently, a laboratorian working with exclusively with a "theoretically" non-infectious LVV was infected with HIV in the absence of any evidence of a laboratory accident.[99]

Insertional Mutagenesis. The second risk associated with LVVs is the potential for insertional mutagenesis or transactivation of neighboring genome sequences. Integration of the transgene can lead to activation of oncogenes, or inactivation of tumor suppressors. Either event can result in promoting the development of cancer. In two separate clinical trials using gamma-retroviral vectors, leukemogenesis was reported in five patients undergoing gene therapy for severe combined immunodeficiency.[100-102] These oncogenic findings were attributed to transcriptional activation of nearby proto-oncogenes by powerful enhancer elements contained within the viral vector.[103]

Worker protections

The risks to workers may increase in the future as synthetic biology is commercialized and proactive steps should be taken now to ensure worker safety as the new field of synthetic biology advances. Worker safety has been a guiding principle of biotechnology since the risks of recombinant DNA were first considered at the 1974 *Asilomar Conference on Recombinant DNA Molecules*.[104] The risk control measures identified then—the use of biological and physical barriers to contain potentially hazardous organisms—remain the mainstay of the largely, self-regulated, voluntary approach to worker protection in synthetic biology today. However, along with the length of genes that can be produced in the laboratory and brought to commercial market,[105] the number and type of workers potentially exposed to "synthetic" genetic engineering processes is rapidly increasing.

The biotechnology sector of the U.S. economy has grown on average greater than 10% each year over the past ten years and the sector is growing much faster that the rest of the economy.[106] Workforce development and workforce protection are both critical in driving the national bioeconomy where economic activity is powered by innovation in the biosciences.[107] Synthetic biology is playing an increasing role in the commercial bioeconomy as providers of biological designs, optimized biological molecules, laboratory suppliers of customer-specified DNA, RNA, enzymes and cell-cloning services, and in drug development. Recent analysis of the private sector landscape shows that 162 U.S companies are engaged in substantial activity in the synthetic biology area with these companies drawing about $6 billion in investments from venture capital individuals and firms.[108] The United Kingdom expects to achieve a market in synthetic biology products equivalent to $10 billion British pound sterling by 2030.[109] The expanding scope of industrial synthetic biology in the new bioeconomy both nationally and internationally marks an opportune time to review existing risk assessment and risk management measures to better protect current and future synthetic biology workers from harm.

Scaling up synthetic biology from laboratory experiments to industrial biofabrication processes requires: enhanced risk governance strategies including disease surveillance, proactive risk assessment involving the larger occupational safety and health practice community beyond research biosafety professionals, the application of prevention-through-design principles to new methods of intrinsic and extrinsic biocontainment and a formal study of their effectiveness, specific synthetic biology guidance for synthetic biology processes used in advanced manufacturing in the new bioeconomy, attention by occupational medicine professionals to viral vector post-exposure, and greater involvement by U.S. national occupational safety and health research and regulatory agencies in ensuring safe approaches to the development of synthetic biotechnology.

Health surveillance

Synthetic biology risk assessment can be enhanced by adding health surveillance capability to current efforts, a need that NIOSH raised in comments to OSHA in 1985.[110] Such surveillance include recording, collecting, and analyzing injury and disease experience of the populations of workers exposed to synthetic biology laboratory, therapeutic and industrial processes. Injury and disease surveillance efforts can be used to minimize potential worker harm. A temporal challenge to disease surveillance in synthetic biology is that many of the adverse oncogenic effects of LVVs and other retroviral vectors may not be detectable for years or decades following exposure. Since workers move from job to job, a long-term exposure registry of synthetic biology workers should be considered. Registries have been successfully used and recommended for other hazardous agents and emerging technologies.[111]

Proactive risk assessment

As synthetic biology emerges from the research laboratory into the bioeconomy, a greater number of occupational safety and health professionals will be involved in ensuring worker safety. The use of synthetic biotechnology in advanced manufacturing requires that occupational safety and health practitioners not currently involved in research biosafety must be educated about risks to workers associated with synthetic biology. More practitioners will have to take a role in proactively assessing the potential risks to workers as synthetic biology products become increasingly used in advanced biological manufacture and in routine clinical care delivery settings. Also, as the synthetic biology workforce expands, worker training tailored to safe approaches to commercial synthetic biology will be needed.

Proactive risk management approaches developed for other emerging technologies such as nanotechnology could be useful in synthetic biology risk assessment and risk management.[112] Proactive risk must involve the freedom for workers to report without fear of reprisal any deviation from high-reliability safety procedures and the duty of employers to conduct a detailed investigation of near-misses and other potential safety failures.[113,114] As applied to other emerging technologies, the proactive approach in synthetic biotechnology provides an opportunity to address occupational health risks at the earliest or design stage prior to widespread dissemination in commercial arenas.[115]

Other aspects of proactive risk management include the identification of industrial scenarios where workers could be exposed to synthetic biological products. Industrial synthetic biology is already a growing field and commercial applications are envisioned in several industries: energy, chemicals, materials, pharmaceuticals, food, and agriculture, as well as in the medical diagnostic and therapeutic areas.[116-118] By identifying the processes where synthetic biology can be used in these industries, risk managers can make proactive assessments of possible risks to workers and what controls need to be put in place.

Little is known about occupational exposures that could occur when synthetic biological products, vectors or organisms are used in industrial scenarios. Exposure assessment involved in synthetic biology will depend on whether the area of interest is in, or around, containment structures or in the external environment. Well-established guidelines have already been developed for pathogenic organisms and recombinant DNA in laboratory research, but not for industrial applications. There is still uncertainty pertaining to the risks associated with the "construction in organisms that may contain genes or proteins that never existed together in a biological organism or that contain newly designed biological functions that do not exist in nature."[69]

Prevention-through design

Applying prevention through design principles to promote further risk control research on intrinsic and extrinsic biocontainment is another avenue to improve worker protection in synthetic biology.[119] The mainstay of risk management for genetic engineering, including for synthetic biology, has been biological containment. Biocontainment can be categorized as extrinsic or intrinsic biocontainment.

Extrinsic biocontainment was developed in the late 1940s and early 1950s chiefly at the U.S. Army Biological Warfare Laboratories at Camp Detrick, Maryland. Extrinsic containment was designed to provide physical containment of highly infectious organisms in secure rooms or cabinets. Biological safety cabinets (BSCs) provide three classes of protection: personal and environmental protection (Class I); personal and environmental protection as well as product protection (Class II); and maximal protection through a gas-tight enclosure where gloves are attached to the front of the BSC to prevent direct contact with hazardous materials (Class III), often referred to as glove boxes.[67] BSCs are now the mainstay of extrinsic biocontainment in laboratories around the world.

Intrinsic biocontainment is a more recent type of containment which takes advantage of the fact that synthetic biology is chiefly an engineering discipline in the life sciences. Intrinsic containment is still under active development in the field of synthetic biology. The aims of intrinsic biocontainment include: (1) controlling growth of the engineered organism in the research laboratory or after an unintentional environmental release; (2) preventing the horizontal flow of genetic material from a synthetic organism to a natural one (gene flow); (3) preventing use of engineered microbes as bioterror agents; and (4) protecting the intellectual property of biotechnology companies.[120] Researchers recognize the need for genetic safeguards intrinsic to the synthetic organism that would restrict its viability in defined environments.[121] Designing these safeguards into synthesized organisms has great promise as a worker protection strategy and aligns well with the emphasis on designing out the hazard to prevent the occurrence of harmful exposures.

Beginning in the 1980s, the field of intrinsic biocontainment has grown rapidly to encompass a number of different strategies. Control of cell growth by engineered auxotrophy, i.e., the inability of an organism to synthesize a particular organic compound required for its growth, protects against environmental release.[122] Intrinsic designs to produce safer viral vectors involve splitting gene vector components into three plasmids, using vectors without viral accessory proteins that are important for a natural virus as a pathogen but not as a vector,[92] and the use of self-inactivating (SIN) vectors which can help mitigate the risk of insertional gene activation.[123,124] Other methods include designing engineered regulators that control expression of essential genes,[125] transcriptional and recombinational strategies to control essential gene function,[125] the use of microbial kill switches,[126] and other vector suicide strategies.[127] The development of an assay that quantitates insertional mutagenesis can help produce safer viral vectors.[128] Finally, a largely theoretical intrinsic biocontainment method involves engineering organisms with chromosomes made not from DNA and RNA, but from xeno ("stranger") nucleic acids or XNA.[129] Unrealistic as such "organisms" seem to us today, their perceived utility would be in preventing "gene flow" with DNA-based organisms, serving as a genetic firewall.

The effectiveness of these intrinsic biocontainment methods and others that emerge in the future should be subjected to rigorous effectiveness studies as synthetic biotechnology becomes more commercial. Funding research on intrinsic biocontainment methods has already been identified as an important need, but funding in this area is not customarily a high priority for governmental biomedical funders or for entrepreneurs.[130] Funding further efforts at both intrinsic and extrinsic biocontainment may be important in advancing worker protection.

Guidance

Risk assessment and management guidance should address the specific risks associated with the rapidly changing issues involved synthetic biology for the general occupational safety and health community. Most safety guidance for working around viral vectors has been generated by private sector research laboratories.[131-134] A *Lentivirus Fact Sheet* was developed through an alliance between OSHA and the American Biological Safety Association, but it chiefly addresses extrinsic biocontainment measures and is dated.[135] Ten years ago, the Recombinant DNA Advisory Committee (RAC), a federal advisory committee that provides recommendations to the NIH Director related to basic and clinical research involving recombinant or synthetic nucleic acid molecules, developed a guidance document called *Biosafety Considerations for Research with Lentiviral Vectors*.[136] The 2006 RAC guidance recommended that a comprehensive risk assessment and containment options should be based on nature of the vector system, transgene insert, and the type of genetic manipulations involved. The guidance further stated that either Biosafety Laboratory (BL), Level 2, or enhanced BL-2, would be appropriate. Since 2006 there has been no recent LVV-specific biosafety refinements or updates from government research or regulatory agencies.

Safety guidance that is specific to synthetic biology should be developed in an electronically updatable format as risk science advances emerge. NIOSH's *Approaches to Safe Nanotechnology* serves as a good example of safety guidance for an emerging technology.[137] Importantly, synthetic biology specific safety guidance should include steps to foster a robust safety culture characterized by employer commitment to safety and worker involvement in ensuring safe synthetic biology. Model guidance could be adopted by national governments as a mandatory standard or used as the basis for a national or international consensus standard.[138-139]

Post-exposure prophylaxis for LVVs

Unintentional exposures to LVVs can potentially occur through inhalation, dermal or gastrointestinal contact, or may occur in the absence of a known exposure incident.[90] The list of workers who could be potentially exposed to blood or other fluids containing LVVs could include researchers, healthcare workers, commercial workers, and support workers such as animal handlers, maintenance and housekeeping workers, and shipping and receiving personnel. Support workers can potentially be exposed to hazardous synthetic biology products or waste when they dispose of products, clean spills, handle contaminated objects, or touch contaminated surfaces.[140]

Primary prevention of LVV exposure incidents is paramount, but if exposure should occur, post-exposure prevention procedures are needed. To date, there are no national or international guidelines about how to handle occupational exposure to HIV-1-derived LVVs as there are for the four other types of pre- and post-exposure interventions—immediate antiretroviral therapy (ART) for the infected partner in a serodiscordant couple, pre-exposure prophylaxis (PrEP), prevention of mother-to-child transmission (PMTCT), and post-exposure prophylaxis (PEP).[141,142] U.S. national guidance for the management of occupational exposures to HIV and post-exposure prophylaxis addresses occupational exposure to wild-type HIV-1 and not to HIV-1-derived LVVs.[143]

Certainly, post-exposure procedures required by OSHA's bloodborne pathogens can be adapted for use after synthetic biotechnology exposures, but details of the appropriate antiretroviral drugs that should be used, their dose, and duration in response to HIV-derived LVV exposure is uncertain. Both infection control and post-exposure prophylaxis guidance is needed to address the infectious and oncogenic risks of unintentional exposure to LVVs in research, healthcare, and industrial settings even though uncertainty exists about the potential risk of insertional mutagenesis from LVV exposure. Despite the current uncertainties about risk, occupational health professionals need to increase their knowledge about synthetic biology as its techniques are scaled up in the larger industrial bioeconomy and worker exposure incidents involving synthetic biology products occur.[144]

Greater awareness and involvement

Synthetic biotechnology presents complex risk assessment and risk management issues to government worker safety and health research and regulatory agencies, and the occupational safety and health practice community. During the research-only phase of synthetic biology, biosafety professionals have worked diligently to keep workers safe. The increase in biosafety research laboratories, the increase in the number of workers potentially exposed to synthetic biology products, the increased use of LVVs and other gene-transfer viral vectors in synthetic biology, and the emerging commercialization of synthetic biology suggest the need for wider involvement by the larger occupational safety and health practice community, and governmental occupational safety and health research and regulatory agencies.

As the *Presidential Commission for the Study of Bioethical Issues* recommended in 2010, greater discussion of risk assessment and risk reduction strategies is an important part of maximizing synthetic biology's benefits and minimizing its harms.[145] As synthetic biology enters more and more workplaces, the need for awareness and involvement by the entire occupational safety and health practice community is needed to ensure the responsible development of commercial synthetic biology.[146]

Conclusion

There is a need to review and enhance current protection measures in the field of synthetic biology, whether in government, academic, or DIY laboratories where new advances are being researched, in health care settings where clinical treatments using pseudotyped viral vector gene delivery systems are increasingly being applied to cancer and immune disorders therapy, or in the expanding industrial bioeconomy where an increasing number of workers will be employed. As the field of synthetic biology grows in importance so will the importance of worker protections to achieve the promised therapeutic and economic benefits of this new field of biotechnology. Advances in biosafety measures to protect synthetic biology workers are growing, but have largely been developed without the active support of U.S. governmental agencies that have worker protection responsibilities as well as the active involvement of the occupational safety and health practice community. Greater involvement by occupational safety and health government agencies, in partnership with synthetic biotechnology researchers and entrepreneurs, and the national and international the biosafety community, would strengthen the current efforts to ensure safer approaches to realizing the exciting promise of synthetic biology.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health, the Centers for Disease Control and Prevention, or the U.S. Department of Health and Human Services.

References

[1] **Ball, P.**: Synthetic biology for nanotechnology. *Nanotechnology 16*:R1–R8 (2005).

[2] **Leduc, S.**: *Theorie Physico-chimique de la vie et Generations Spontanees*. Paris: A. Poinat, 1910. p. 202.

[3] **Szybalski, W.**: In vivo and in vitro initiation of transcription. In *Control of Gene Expression*. A. Kohn and A. Shatkay (Eds.). New York: Plenum Press, 1974. pp. 23–4, 404–5, 411–2, and 415–7.

[4] **Syzbalski, W., and A. Skalka**: Nobel prizes and restriction enzymes. *Gene 4*:181–182 (1978).

[5] **Ad Hoc Technical Expert Group on Synthetic Biology**: “Report of the Ad Hoc Technical Expert Advisory Group on Synthetic Biology. Convention on Biological Diversity.” Available at https://www.cbd.int/doc/meetings/synbio/synbioahteg-2015-01/official/synbioahteg-2015-01-03-en.pdf (accessed May 13, 2016).

[6] **Baker, D., G. Church, J. Collins, et al.**: Engineering life: building a FAB for biology. *Scientific Am. 294*:44–51 (2006).

[7] **Eisenstein, M.**: Living factories of the future. *Nature 531*:401–403 (2016).

[8] **Smith, H.O., C.A. Hutchison, C. Pfannkoch, and J.C. Venter**: Generating a synthetic genome by whole genome assembly: ϕX174 bacteriophage from synthetic oligonucleotides. *PNAS 100*(26):15440–15445 (2003).

[9] **Gibson, D.G., G.A. Benders, C. Andrews-Pfannkoch, et al.**: Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. *Science 319*:1215–1219 (2008).

[10] **Gibson, D.G., J.I. Glass, C. Lartigue, et al.**: Creation of a bacterial cell controlled by a chemically synthesized genome. *Science 329*:52–56 (2010).

[11] **Miller, D.M., and J.M. Gulbis**: Engineering protocells: prospects for self-assembly and nanoscale production lines. *Life 5*:1019–1053 (2015).

[12] **Kurihara, K., Y. Okura, M. Matsuo, T. Toyota, K. Suzuki, and T. Sugawara**: A recursive vesicle-based model protocell with a primitive model cell cycle. *Nature Comm. 6*:1–7 (2015).

[13] **Pinheiro, V.B., and P. Holliger**: The XNA world: progress toward replication and evolution of synthetic genetic polymers. *Curr. Opin. Chem. Biol. 16*(3-4):245–252 (2012).

[14] **Synthetic Biology Project:** “What is Synthetic Biology?” Available at http://www.synbioproject.org/topics/synbio101/definition/ (accessed May 15, 2016).

[15] **Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, and E. Charpentier**: A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science 337*:816–821 (2012).

[16] **Long, C., L. Amoasii, A.A. Mireault, et al.**: Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science 351*:400–403 (2016).

[17] **Nelson, C.E., C.H. Hakim, D.G. Ousterout, et al.**: In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science 351*(6271):403–407 (2016).

[18] **Tabebordbar, M., K. Zhu, J.K.W. Cheng, et al.**: In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science 351*: 407–411 (2016).

[19] **Yin, H., W. Xue, S. Chen, et al.**: Genome editing with a Cas9 in adult mice corrects a disease mutation and phenotype. *Nature Biotechnol. 32*(6):551–553 (2014).

[20] **Kaminski, R., Y. Chen, T. Fischer, et al.**: Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. *Sci. Rep. 6*:6–14 (2016).

[21] **Naldini, L.**: Gene therapy returns to centre stage. *Nature 526*:351–360 (2015).

[22] **Van den Belt, H.**: Playing God in Frankenstein’s footsteps: Synthetic biology and the meaning of life. *Nanoethics 3*:257–268 (2009).

[23] **Boldt, J.**: Creating life: synthetic biology and ethics. In *Synthetic Biology and Morality*, **G.E. Kaebnick and T.H. Murray** (eds.). Cambridge, MA: The MIT Press, 2013. pp. 35–49.

[24] **Garfinkel, M.S., D. Endy, G.L. Epstein, and R.M. Friedman**: “Synthetic Biology.” Available at http://www.thehastingscenter.org/uploadedFiles/Publications/Briefing_Book/synthetic%20biology%20chapter.pdf (accessed April 26, 2016).

[25] **Fukuyama, F.**: “Transhumanism.” Available at http://foreignpolicy.com/2009/10/23/transhumanism/ (accessed April 30, 2016).

[26] **Tumpey, T.M., C.F. Basler, P.V. Aguilar, et al.**: Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science 310*:77–90 (2005).

[27] **Sharples, F., J. Husbands, A-M. Mazza, A. Thevenon, and I. Hook-Barnard**: *Potential Risks and Benefits of Gain-of-function Research: Summary of a Workshop*. Washington, DC: National Academies of Sciences, Engineering and Medicine, 2015.

[28] **DIYBIO**. An institution for the do-it-yourself biologist. 2016. Available at https://diybio.org/ (accessed May 14, 2016).

[29] **Church, G., and E. Regis**: *Regeneris: How Synthetic Biology will Reinvent Nature and Ourselves*. New York: Basic Books, 2012.

[30] **Pollack, A.**: "Scientists Talk Privately about Creating a Synthetic Human Genome." Available at http://www.nytimes.com/2016/05/14/science/synthetic-human-genome.html (accessed May 14, 2016).

[31] **Lander, E.S.**: Brave new genome. *New Engl. J. Med. 373*(1):5–8 (2015).

[32] **Hayden, E.C.**: Tech investors bet on synthetic biology. *Nature 527*:19 (2015).

[33] **Kuzma, J.**: Reboot the debate on genetic engineering. *Nature 531*:165–167 (2016).

[34] **Young, A., and N. Penzenstadler**: "Inside America's Secretive Biolabs." Available at http://www.usatoday.com/story/news/2015/05/28/biolabs-pathogens-location-incidents/26587505/ (accessed April 30, 2016)

[35] **Young, A.**: "CDC Labs Repeatedly Faced Secret Sanctions for Mishandling Bioterror Germs." Available at http://www.usatoday.com/story/news/2016/05/10/cdc-lab-secret-sanctions/84163590/ (accessed May 12, 2016).

[36] **Pollack, A., and D. Wilson**: "Safety Rules Can't Keep Up with Biotech." Available at http://www.nytimes.com/2010/05/28/business/28hazard.html?_r=0

[37] **Liang, P., Y. Xu, X. Zhang, et al.**: CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein Cell* 6(5):363–372 (2015).

[38] **Cyranoski, D., and S. Reardon**: Embryo editing sparks epic debate. *Nature 620*:593–594 (2015).

[39] **Baltimore, D., P. Berg, M. Botchan, et al.**: A prudent path forward for genomic engineering and germline gene modification. *Science 348*:36–38 (2015).

[40] **Lanphier, E., F. Urnov, S.E. Haecker, M. Werner, and J. Smolenski**: Don't edit the human germline. *Nature 519*:410–411 (2015).

[41] **Pennisi, E.**: "111 Organizations Call for Synthetic Biology Moratorium." Available at http://www.sciencemag.org/news/2012/03/111-organizations-call-synthetic-biology-moratorium (accessed May 15, 2016).

[42] **African Biodiversity Network (Kenya), African Centre for Biosafety (South Africa), Alliance for Humane Biotechnology, et al.**: "Civil Society Letter to President's Commission on Synthetic Biology." Available at http://www.geneticsand-society.org/article.php?id=5517 (accessed May 11, 2016).

[43] **U.S. Department of Health and Human Services**: "Screening Framework Guidance for Providers of Synthetic Double-stranded DNA." Available at http://www.phe.gov/Preparedness/legal/guidance/syndna/Documents/syndna-guidance.pdf (accessed April 30, 2016).

[44] **U.S. Department of Health and Human Services**: "Dual Use Research of Concern." Available at http://www.phe.gov/s3/dualuse/Pages/default.aspx (accessed June 30, 2016).

[45] **Tucker, J.B.**: Could terrorists exploit synthetic biology? *The New Atlantis*, Spring 2011.

[46] **Tucker, J.B., and R.A. Zilinskas**: The promise and perils of synthetic biology. *The New Atlantis*, Spring, 2006.

[47] **International Risk Governance Council**: *Guidelines for the Appropriate Risk Governance of Synthetic Biology*. Available at http://www.irgc.org/IMG/pdf/irgc_SB_final_07jan_web.pdf (accessed April 22, 2016).

[48] **Office of Science Technology and Policy (OSTP)**: *Coordinated Framework for Regulation of Biotechnology*. Available at http://www.aphis.usda.gov/brs/fedregister/coordinated_framework.pdf (accessed April 28, 2016).

[49] **Code of Federal Regulations**: "Bloodborne Pathogens." Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051 (accessed May 14, 2016).

[50] **Code of Federal Regulations**: "Toxic and Hazardous Substances." Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=9991&p_table=STANDARDS (accessed May 14, 2016).

[51] **Code of Federal Regulaions**: "Access to Employee Exposure and Medical Records." Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10027 (accessed May 14, 2016).

[52] **Code of Federal Regulaions**: "Hazard Communication." Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10099 (accessed May 14, 2016).

[53] **Code of Federal Regulaions**: "Occupational Exposure to Hazardous Chemicals in Laboratories." Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10106 (accessed May 14, 2016).

[54] **Code of Federal Regulaions**: "Respiratory Protection." Available at https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=12716 (accessed May 14, 2016).

[55] "Exercise of Federal Oversight within Scope of Statutory Authority: Planned Introductions of Biotechnology Products into the Environment," *Federal Register 57*:39 (February 27, 1992). pp. 6753–6762.

[56] **Holdren, J., H. Shlanski, D. Vetter, and C. Goldfuss**: "Modernizing the regulatory system for biotechnology products." Available at https://www.whitehouse.gov/sites/default/files/microsites/ostp/modernizing_the_reg_system_for_biotech_products_memo_final.pdf (accessed May 14, 2016).

[57] **Barbero, R., and J. Kim**: "Clarifying Current Roles and Responsibilities for Biotechnology Products." Available at https://www.whitehouse.gov/blog/2016/03/

07/clarifying-current-roles-and-responsibilities-biotechnology-products (accessed April 30, 2016).

[58] **Ledford, H.**: Gene editing surges as U.S. rethinks regulations. *Nature 532*:158–159 (2016).

[59] **Occupational Safety and Health Administration (OSHA)**: "Infectious Disease Rulemaking." Available at https://www.osha.gov/dsg/id/ (accessed May 14, 2016).

[60] **Bergeson, L.L., L.M. Campbell, S.L. Dolan, et al.**: "*The DNA of the U.S. Regulatory System: Are We Getting it Right for Synthetic Biology?*" Available at http://www.synbioproject.org/publications/dna-of-the-u.s-regulatory-system/ (accessed on May 10, 2016).

[61] **Carter, S.R., M. Rodemeyer, M.S. Garfinkel, and R. Friedman**: "Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options." Available at http://www.jcvi.org/cms/research/projects/synthetic-biology-and-the-us-biotechnology-regulatory-system/overview/ (accessed May 9, 2016).

[62] **J. Craig Venter Institute**: *Synthetic Biology and the U.S. Biotechnology Regulatory System: Challenges and Options.* La Jolla, CA: J. Craig Venter Institute, 2014.

[63] **Lowrie, H., and J. Tait**: "Policy Brief: Guidelines for the Appropriate Risk Governance of Synthetic Biology." Available at http://www.irgc.org/IMG/pdf/irgc_SB_final_07jan_web.pdf(accessed May 10, 2016).

[64] **Friends of the Earth U.S., International Center for Technology Assessment, and ETC Group**: "The Principles for the Oversight of Synthetic Biology, 2010." Available at http://www.synbioproject.org/process/assets/files/6620/_draft/principles_for_the_oversight_of_synthetic_biology.pdf (accessed May 10, 2016).

[65] **Garfinkel, M.S., D. Endy, G.L. Epstein, and R.M. Friedman**: "Synthetic Genomics: Options for Governance." Available at http://www.synbiosafe.eu/uploads/pdf/Synthetic%20Genomics%20Options%20for%20Governance.pdf (accessed May 14, 2016).

[66] **World Health Organization (WHO)**: "Laboratory Biosafety Manual. 3rd ed." Available at http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1 (accessed May 14, 2016).

[67] **U.S. Department of Health and Human Services**: "Biosafety in Microbiological and Biomedical Laboratories, 5th ed." Available at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm (accessed December 23, 2015).

[68] **National Science Advisory Board for Biosecurity (NSABB)**: "Addressing Biosecurity Concerns related to Synthetic Biology." Available at http://osp.od.nih.gov/sites/default/files/resources/NSABB%20SynBio%20DRAFT%20Report-FINAL%20%282%29_6-7-10.pdf (accessed on May 14, 2016)

[69] **National Institutes of Health (NIH)**: "NIH Guidelines for Research involving Recombinant or Synthetic Nucleic Acid Molecules." Available at http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines (accessed May 14, 2016).

[70] **U.S. Department of Health and Human Services**: "United States Government Policy for Institutional Oversight of Life Science Dual Use Research of Concern." Available at http://www.phe.gov/s3/dualuse/documents/durc-policy.pdf (accessed June 30, 2016).

[71] **Bailey, C., H. Metcalf, and B. Crook**: "Synthetic Biology: A Review of the Technology, and Current and Future Needs from the Regulatory Framework in Great Britain." Available at http://www.hse.gov.uk/research/rrpdf/rr944.pdf (accessed May 22, 2016).

[72] **Pauwels, K, N. Willemarck, D. Breyer, and P. Herman**: "Synthetic Biology: Latest Developments, Biosafety Considerations and Regulatory Challenges." Available at http://www.biosafety.be/PDF/120911_Doc_Synbio_SBB_FINAL.pdf (accessed May 22, 2016).

[73] **Netherlands Commission on Genetic Modification**: "Biological Machines? Anticipating Developments in Synthetic Biology." Available at: http://www.cogem.net/index.cfm/en/publications/publication/biological-machines-anticipating-developments-in-synthetic-biology (accessed November 15, 2016).

[74] **European Commission (EC)**: "Opinion on Synthetic Biology II: Risk Assessment Methodologies and Safety Aspects. European Union." Available at http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_048.pdf (accessed May 12, 2016).

[75] **Breitling, R., E. Takano, and T.S. Gardner**: Judging synthetic biology risks. *Science 347*;107 (2015).

[76] **Bar-Yam, S., J. Byers-Corbin, R. Casagrande, et al.**: "The Regulation of Synthetic Biology: A Guide to United States and European Union Regulations, Rules And Guidelines." Available at https://www.synberc.org/sites/default/files/Concise%20Guide%20to%20Synbio%20Regulation%20OYE%20Jan%202012_0.pdf (accessed May 14, 2016).

[77] **Tucker, J.B.**: Introduction. In *Innovation, Dual-Use and Security: Managing the Risks of Emerging Biological and Chemical Technologies*, J.B. Tucker (ed.) Cambridge, MA: MIT Press, 2012. pp. 4–6.

[78] **Cong, L., F.A. Ran, S. Cox, et al.**: Multiplex genome engineering using CRISPR/Cas systems. *Science 339*:819–823 (2013).

[79] **McNutt, M.**: Breakthrough to genome editing. *Science 350*:1445 (2015).

[80] **Ledford, H.**: CRISPR, the disruptor. *Nature 522*:20–24 (2015).

[81] **Travis, J.**: Making the Cut: CRISPR genome-editing technology shows its power. *Science 350*:1456–1457 (2015).

[82] **Cookson, C.**: UK Scientists given green light to 'edit' genes in human embryos. *Financial Times*, February 1, 2016.

[83] **Blaese, R.M., K.W. Culver, A.D. Miller, et al.**: T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. *Science 270*: 475–480 (1995).

[84] **Cross, D., and J.K. Burmester**: Gene therapy for cancer treatment: Past, present and future. *Clin. Med. Res., 4*(3):218–227 (2006).

[85] **Merten, O-W, and M. Al-Rubeai**: *Viral Vectors for Gene Therapy*. New York: Human Press, 2011.

[86] **Robbins, P.D., and S.C. Ghivizzani**: Viral vectors for gene therapy. *Pharmacol. Ther. 80*(1): 35–47 (1998).

[87] **Levine, B.L., L.M. Humeau, J. Boyer, et al.**: Gene transfer in humans using a conditionally replicating lentiviral vector. *PNAS 103*(46):17372–17377 (2006).

[88] **Maetzig, T., M. Galla, C. Baum, and A. Schambach**: Gammaretroviral vectors: Biology, technology and application. *Viruses 3*:677–713 (2011).

[89] **Tomas, H.A., A.F. Rodriquez, P.M. Alves, and A.S. Corodinha**: "Lentiviral Gene Therapy Vectors: Challenges and Future Directions." Available at http://www.intechopen.com/books/gene-therapy-tools-and-potential-applications/lentiviral-gene-therapy-vectors-challenges-and-future-directions (accessed May 13, 2016).

[90] **Howard J.**: HIV infection in the workplace. *The Occupational and Environmental Medicine Report 4*(8):60–64 (1990).

[91] **Beckwith, C.G., E.J. Wing, B. Rodriguez, and M.M. Lederman**: Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome. In *Cecil Essentials of Medicine*, T.E. Andreoli, I. Benjamin, R.C. Griggs, and E.J. Wing (eds.). Philadelphia, PA: Saunders, 2010. pp. 1008–1027.

[92] **Sakuma, T, M.A. Barry, and Y. Ikeda**: Lentiviral vectors: basic to translational. *Biochem. J. 443*:603–618 (2012).

[93] **Journal of Gene Medicine**: "Gene Therapy Clinical Trials Worldwide." Available at http://www.wiley.com/legacy/wileychi/genmed/clinical/ (accessed May 13, 2016).

[94] **Pauwels, K., R. Gijsbers, J. Toelen, et al.**: State-of-the-art lentiviral vectors for research use: risk assessment and biosafety recommendations. *Curr. Gene Ther. 9*:459–474 (2009).

[95] **Cronin, J., X-Y Zhang, and J. Reiser**: Altering the tropism of lentiviral vectors through pseudotyping. *Curr. Gene Ther.* 5(4):387–398 (2005).

[96] **Schambach, A., D. Zychlinski, B. Erhnstroem, and C. Baum**: Biosafety features of lentiviral vectors. *Hum. Gene Ther. 24*:132–142 (2013).

[97] **Mosier, D.E.**: Introduction for 'Safety considerations for retroviral vectors: a short review'. *Appl. Biosaf. 9*(2):68–75 (2004).

[98] **Wright, O., G-B. Stan** and **T. Ellis**: Building-in biosafety for synthetic biology. *Microbiology 159*:1221–1235 (2013).

[99] **Alteri, C., A. Soria, A. Bertoli, et al.**: "HIV-1 Laboratory Contagion during Recombination Procedures with Defective Constructs." Available at http://www.croi-conference.org/sessions/hiv-1-laboratory-contagion-during-recombination-procedures-defective-constructs (accessed April 25, 2016).

[100] **Hacen-Bey-Abina, S., C. Von Kalle, M. Schmidt, et al.**: LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science 302*:415–419 (2003).

[101] **Hacen-Bey-Abina, S., A. Garrigue, and G.P. Wang**: Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest. 118*(9):3132–3142 (2008).

[102] **Howe, S.J., M.R. Mansour, and K. Schwarzwaelder**: Insertional mutagenesis combined with somatic mutations causes leukemogensis following gene therapy of SCID-X1 patients. *J Clin. Invest. 118*(9):3143–3250 (2008).

[103] **Persons, D.A.**: Lentiviral vector gene therapy: effective and safe? *Molec. Ther. 18*(5): 861–862 (2010).

[104] **Berg, P., D. Baltimore, S. Brenner, R.O. Roblin III, and M.F. Singer**: Summary statement of the Asilomar Conference on Recombinant DNA Molecules. *PNAS 72*(6):1981–1984 (1975).

[105] **LaVan, D.A., and L.M. Marmon**: Safe and effective synthetic biology. *Nat. Biotech. 28*(10):1010–1012 (2010).

[106] **Carlson, R.**: Estimating the biotech sector's contribution to the US economy. *Nat. Biotech. 34*(3):247–255 (2016).

[107] **White House**: "National Bioeconomy Blueprint." Available at https://www.whitehouse.gov/sites/default/files/microsites/ostp/national_bioeconomy_blueprint_april_2012.pdf (accessed May 10, 2016).

[108] **U.S. Department of Defense (DOD)**: "Technical Assessment: Synthetic Biology." Available at http://defenseinnovationmarketplace.mil/resources/OTI-SyntheticBiologyTechnicalAssessment.pdf (accessed May 22, 2016)

[109] **Synthetic Biology Leadership Council**: "Biodesign for the Bioeconomy: UK Synthetic Biology Strategic Plan 2016." Available at http://www.synbio.cam.ac.uk/news/synbio-strategic-plan-2016 (accessed July 2, 2016).

[110] "Biotechnology," *Federal Register 50*:12 (12 April 1985). pp. 14468–14470.

[111] **Schulte, P., D.J. Mundt, M. Nasterlack, K.B. Mulloy, and K.A. Mundt**: Exposure registries: overview and utility for nanomaterial workers. *J Occup Environ Med 53*(6 Suppl.):S42–47 (2011).

[112] **Murashov, V., and J. Howard**: Essential features of proactive risk management. *Nat. Nanotechnol. 4*: 467–470 (2009).

[113] **Weick, K.E., Sutcliffe, K.M.**: *Managing the Unexpected: Sustained Performance in a Complex World*. Hoboken, NJ: Wiley and Sons, 2015.

[114] **Trevan, T.**: Biological research: rethink biosafety. *Nature 527*:155–158 (2015).

[115] **Schulte, P., R. Rinehart, A. Okun, G.L. Geraci, and D.S. Heidel**: National prevention through design (PtD) initiative. *J Safety Res 39*(2):115–121 (2008).

[116] **Erickson, R., H.R. Sing, and P. Winters**: Synthetic biology: regulating industry uses of new biotechnologies. *Science 333*:1254–1256 (2011).

[117] **Schmidt, M.**: Introduction. In *Synthetic Biology: Industrial and Environmental Applications*, M. Schmidt (ed.). Weinheim, Germany: Wiley-Blackwell, 2012. pp. 1–6.

[118] **Rohn, J.**: Synthetic biology goes industrial. *Nat. Biotechnol. 31*:773 (2013).

[119] **National Institute for Occupational Safety and Health (NIOSH)**: "The State of the National Initiative on Prevention through Design. Centers For Disease Control And Prevention." Available at http://www.cdc.gov/niosh/docs/2014-123/pdfs/2014-123.pdf (accessed May 14, 2016)

[120] **Cai, Y., N. Agmon, W.J. Choi, et al.**: Intrinsic biocontainment: multiplex genome safeguards combine transcriptional and recombinational control of essential yeast genes. *PNAS 112*(6): 1803–1808 (2015).

[121] **Schmidt, M., and V. de Lorenzo**: Synthetic constructs in/for the environment: managing the interplay between

natural and engineered biology. *FEBS Letters 586*:2199–2206 (2012).

[122] **Steidler, L., S. Neirynck, N. Huyghebaert, et al.**: Biological containment of genetically modified Lactococcus lactis for intestinal delivery of human interleukin 10. *Nat. Biotechnol. 21*:785–789 (2003).

[123] **Cockrell, A.S., H. Ma, K. Fu, T.J. McCown, and T. Kafri**: A trans-lentiviral packaging cell line for high-titer conditional self-replicating HIV-1 vectors. *Molec. Ther. 14*(1):276–284 (2006).

[124] **Zufferey, R., T. Dull, R.J. Mandel, et al.**: Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J. Virol. 72*(12):9873–9880 (1998).

[125] **Gallagher, R.R., J.R. Patel, A.L. Interiano, A.J. Rovner, and F.J. Isaacs**: Multilayered genetic safeguards limit growth of microorganisms to defend environments. *Nucl. Acids Res. 43*(3):1945–1954 (2015).

[126] **Chan, C.T.Y., J.W. Lee, E. Cameron, C.J. Bashor, and J.J. Collins**: 'Deadman' and 'passcode' microbial kill switches for bacterial containment. *Nat. Chem. Biol. 12*:82–86 (2016).

[127] **Bej, A.K., M.H. Perlin, and R.M. Atlas**: Model suicide vector for containment of genetic engineered microorganisms. *Appl. Environ. Microbiol. 54*(10):2472–2477 (1988).

[128] **Bokhoven, M., S.L. Stephen, S. Knight, et al.**: Insertional gene activation by lentiviral and gammaretroviral vectors. *J. Virol. 83*(1):283–294 (2009).

[129] **Schmidt, M.**: Xenobiology: A new form of life as the ultimate biosafety tool. *BioEssays 32*(4):322–331 (2010).

[130] **Garfinkel, M.**: "Biological Containment of Synthetic Microorganisms: Science and Policy." Available at http://www.embo.org/documents/science_policy/biocontainment_ESF_EMBO_2012_workshop_report.pdf (accessed April 30, 2016).

[131] "Working With Viral Vectors." Available at https://web.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf (accessed May 11, 2016).

[132] **Biosafety Office:** "Lentiviral Vectors." Available at http://research-compliance.uc.edu/training/lentiviral-vectors/story_content/external_files/LV%20online%20%28March%202014b%29.pdf (accessed May 15, 2016).

[133] **Gray, J.T.**: Laboratory safety for oncogene-containing retroviral vectors. *Appl. Biosaf. 16*(4):218–222 (2011).

[134] **Byers, K.B.**: Biosafety tips. *Appl. Biosaf. 20*:250–252 (2015).

[135] **American Biological Safety Association (ABSA)**: "Lentivirus Vector Fact Sheet." Available at https://www.absa.org/pdf/LentivirusVectorFactSheet.pdf (accessed April 21, 2016).

[136] **National Institutes of Health (NIH)**: "Biosafety Considerations for Research With Lentiviral Vectors." Available at http://osp.od.nih.gov/sites/default/files/resources/Lenti_Containment_Guidance_0_0.pdf (accessed on May 12, 2016).

[137] **National Institute for Occupational Safety and Health (NIOSH)**: "Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated With Engineered Nanomaterials." Available at http://www.cdc.gov/niosh/docs/2009-125/pdfs/2009-125.pdf (accessed May 12, 2016).

[138] **Murashov V., and J. Howard**: The U.S. must help set international standards for nanotechnology. *Nat. Nanotechnol. 3*:635–636 (2008).

[139] **Murashov, V., P. Schulte, C. Geraci, and J. Howard**: Regulatory approaches to worker protection in nanotechnology industry in the USA and European Union. *Industr. Health 49*:280–296 (2011).

[140] **Murashov, V., and J. Howard**: Risks to health care workers from nano-enabled medical products. *J. Occ. Env. Hyg*. 12(6):D75–D85 (2015).

[141] **Baggaley, R., M. Doherty, A. Ball, N. Ford, and G. Hirnschall**: The strategic use of antiretrovirals to prevent HIV infection: A converging agenda. *Clin. Infect. Dis. 60*(Supp 3):S159–S160 (2015).

[142] **Centers for Disease Control and Prevention (CDC)**: "Updated Guidelines for Antiretroviral Postexposure Prophylaxis After Sexual, Injection Drug Use, or Other Nonoccupational Exposure to HIV—United States, 2016." Available at http://www.cdc.gov/hiv/pdf/programresources/cdc-hiv-npep-guidelines.pdf (accessed May 12, 2016).

[143] **Kuhar, J.T., D.K. Hendersen, K.A. Struble, et al.**: "Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis" Available at https://stacks.cdc.gov/view/cdc/20711 (accessed May 14, 2016).

[144] **Warnock, J.N., C. Daigre, and M. Al-Rubeai**: Introduction to viral vector. In *Viral Vectors*, O-W. Merten and M. Al-Rubeai (Eds.), New York: Humana Press. p. 1.

[145] **Presidential Commission for the Study of Biomedical Issues**: "New Directions: The Ethics of Synthetic Biology and Emerging Technologies." Available at http://bioethics.gov/synthetic-biology-report (accessed May 21, 2016).

[146] **Moe-Behrens, G.H.G, R. Davis, and K.A. Haynes**: Preparing synthetic biology for the world. *Front. Microbiol. 4*(5):1–10 (2013).