TERROR
Strengthened preparedness and response to biological and chemical terror attacks

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WP8 Novel Threats

WP8.1: State of the art of Synthetic Biology- Literature Review

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Terms and abbreviations

AG	Australia Group
AI	Artificial Intelligence
AMR	Antimicrobial Resistance
ARG	Antimicrobial Resistance Gene
DURC	Dual Use Research of Concern
DIY biology	Do-It-Yourself biology
CBRN	Chemical, Biological, Radiological or Nuclear
CRISPR/Cas/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats. Cas stands for "CRISPR associated" and refers to a group of nucleases such as Cas9 which is an RNA guided nuclease widely used in gene editing.
Desktop DNA/RNA synthesizers	Desktop machines that can synthesize fragments of DNA/RNA
Genomics	Study of the total or part of the genetic or epigenetic sequence information of organisms.
Gibson Assembly	Molecular technique which allows the joining of multiple DNA fragments. Named after its inventor Daniel G. Gibson.
GoF research	Gain-of-Function research refers to genomic alterations in organisms to enhance biological functions, e.g., pathogenesis, type of host, etc.
Hybrid warfare	Warfare which involves an interplay or fusion of conventional as well as unconventional instruments of power and tools of subversion
IGSC	International Gene Synthesis Consortium
LoF research	Loss-of-Function research refers to genomic alterations in organisms that result in a decrease of biological functions e.g., decreasing a pathogen's ability to cause disease.
Metagenomics	Study of the structure and function of entire nucleotide sequences isolated and analyzed from all the organisms (typically microbes) in a bulk sample.
MCMs	Medical Countermeasures
NGS	Next Generation Sequencing
Phylogenomics	The study of evolutionary relationships among organisms based on the comparison of their entire genomes
TALEN	Transcription Activator-Like Effector Nucleases
WGS	Whole Genome Sequencing

Executive summary

This review explores recent technological advancements in synthetic biology. Developments within this field bring a wealth of potential benefits to multiple sectors, including health, energy, agriculture, and private industry. However, developments also bring risks, particularly in the context of potential bioterror attacks or biological warfare. We have focussed this review on three areas: the creation and/or adaption of organisms which we describe under the heading genetic engineering; the large-scale or novel production of proteins, peptides or genetic material which is described under the heading heterologous protein expression and mRNA technology; and finally, the role of whole genome sequencing (WGS) and the open access data it produces that underscore many of the technologies examined in this review. The convergence of these technologies underscores a central theme of dual use, where advancements driving progress can be exploited for malicious purposes, including bioweapon creation.

Genetic engineering methods such as CRISPR/Cas offer huge potential to the fields of medicine and agriculture. With these techniques it is possible to cure hereditary diseases, diagnose and treat mental illness or make crops resistant to the impacts of climate change. However, it is also possible to create highly pathogenic agents or to modify existing pathogens to enhance their virulence, alter host ranges, or confer resistance to treatments. It is this dual nature coupled with the development of simpler methods, commercially available kits, and the growth of private or even 'DIY' laboratories that raises serious concerns.

Advances in heterologous protein expression and mRNA technology have revolutionized medical and biotechnological landscapes. However, their democratization raises concerns about unintended harm or malicious applications, especially the potential nefarious design and production of harmful effector proteins. The transformative efficacy of mRNA technology, exemplified by COVID-19 vaccines, comes with huge potential but these advancements come with inherent risks that merit meticulous attention in the context of bioweapon threats.

WGS plays a critical role in bioterrorism response. However, the potential misuse of genetic information held in publicly available databases introduces concerns, emphasizing the need for robust biosecurity measures to guard against cyber and bio-attacks. While it significantly advances our understanding of pathogenic traits and aids in disease surveillance, its implications for bioterrorism are concerning.

Literature reviews provide a snapshot of an area of science at a certain moment in time. They can indicate future directions and forewarn us of potential threats. While we describe the technologies within this review as novel, we remind readers that novelty is fleeting and may be commonplace by the time of publication. This area requires constant surveillance and re-examination to remain novel. Our last search took place in February 2022 and much has changed even in this short period. Artificial Intelligence (AI) is dominating conversations as this review is being finalised and it is the opinion of the authors that this will accelerate scientific advancements, in ways that are yet to be seen.

This review does not aspire to be a fully comprehensive. There are numerous ongoing technological advancements in synthetic biology and making a complete list of developments within synthetic biology is beyond the reach of this report. Thus, the developments described are just examples and not a complete picture of the technological advancements in the field.

We found no examples of synthetic biology being accidentally or deliberately used to cause harm. On the other hand, examples of synthetic biology being used to recreate or modify highly pathogenic agents for beneficial or research purposes, clearly demonstrates what is technically possible.

Improved risk assessments and expanded use of safety laboratories for experiments where dual-use risks are assessed to exist should result in increased biosecurity. Even though increased volume and a higher number of researchers using synthetic biology techniques inherently increases the risk of unforeseen events occurring, whether due to accidents, ignorance, poor planning, or negligence. This risk cannot be completely mitigated but must be weighed against the potentially beneficial outcomes of the pursued research and the contribution it might make to the common good. In this context, a mitigating factor is that most experiments conducted with good intentions are not designed to optimize the fitness of the microorganisms outside the laboratory environment. Biological competition and environmental factors to which the organisms are not adapted to, will probably reduce the consequences in most cases. However, if experiments are conducted with the purpose of causing harm and are carried out covertly, the risks increase significantly. It is practically impossible to detect intent, as the development or production of dangerous agents can be done on a small scale and decentralized, making it easier to conceal

There is an ongoing debate about what oversights, regulations, and potentially legislative provisions are needed to mitigate potential risks emanating from synthetic biology research and associated technological developments. However, a fragmented landscape of national frameworks, various

formal international organizations and informal multinational groups with different governance and oversight frameworks results in unclear responsibility areas between different actors. Therefore, significant benefits could be gained from a more harmonized approach to biorisk management and standardization concerning training and reporting on potential biorisks. Further, a greater engagement, communication and awareness among scientist, policymakers and stakeholders including the public, is needed to ensure that synthetic biology is developed and used in a safe, responsible, and transparent manner.

Within academia and research, there is a need for greater education and workforce development to ensure that there is a skilled and diverse workforce to drive innovation in this field. Education programs should also include awareness raising on ethical and legal risks. This will require the development of new training programs, as well as efforts to increase diversity and inclusion in the field.

Given the widespread availability of knowledge and technology in the field, it is essential to be prepared for the possibility of an incident by having well-developed healthcare systems, efficient diagnostic tools, and a strong foundation of basic research in relevant areas. By building a strong foundation of knowledge and capabilities in these areas, we can minimize the impact of incidents and help to ensure the health and safety of individuals and communities around the world, while also continuing to improve biosecurity and biosafety measures to prevent incidents from occurring in the first place.

Introduction

Joint Action TERROR

The European Union (EU) plays an important role in counter-terrorism activities. While primary responsibility for security measures lies with individual Member States, the EU provides a borderless perspective that encourages cooperation and coordination through numerous policy frameworks.

EU Regulation 2022/2371 (Council of the European Union, 2022) seeks to build a stronger EU health security framework by improving coordination between the European Commission and other EU

agencies. The regulation was formally adopted during the lifecycle of Joint Action TERROR and repeals Decision No 1082/2013/EU on serious cross-border threats to health. It provides the framework to improve preparedness and to strengthen the response capacities to health emergencies of biological, chemical, environmental, and unknown origin.

The 2009 Commission Working document 'Bridging Security and Health' identified areas that could be strengthened. It states, among other issues, that Member States preparedness in health would benefit from sharing lessons learned and best practices in, among other issues, cross-sectoral support, and coordination.

To support this, Joint Action TERROR's main objectives were to address gaps in health preparedness and to strengthen cross-sectoral work with security, civil protection, and health sectors response to biological and chemical terror attacks.

Joint Action TERROR aimed to build upon work undertaken for the Health Programme and other relevant EU programmes and exercises in particular Joint Action "Strengthened International Health Regulations and Preparedness in the EU" (SHARP) and the Joint Action "Healthy Gateways".

Bioterrorism

Terrorist attacks usually occur without warning which poses significant challenges for health preparedness and cross sectoral response planning. The unpredictable nature of terrorism, coupled with its potential impact on individuals and communities, adds complexity to preparedness and response strategies. These intentional acts can encompass a range of tactics and weaponry, including both conventional and non-conventional means such as chemical, biological, radioactive, and nuclear (CBRN) materials, as well as hybrid elements and cyber-attacks (WHO, 2023). Effective coordination of cross-sectoral responses is crucial, particularly during multi-site events. According to NATO, the potential use of CBRN agents or weapons of mass destruction (WMD), by state or on-state actors, pose a central and evolving security threat (NATO, 2022). Such attacks possess the potential to swiftly escalate, cause significant loss of life or harm to individuals, strain healthcare capabilities, disrupt government and public services, and cause substantial economic repercussions. Additional difficulties can arise from covert attacks as critical details may be obscured and cause delays to appropriate responses. Regardless, when assessing risks and preparing for incidents resulting from a terrorist attack, it is essential to acknowledge that these events can occur overtly or covertly.

Biological weapons are prohibited according to the Biological Weapons Convention (BWC) which entered into force on 26 March 1975 and currently has 185 State Parties and four Signatory States (UNODA, 2023). However, the convention lacks verification mechanisms and is claimed to have been violated several times (Dahlburg, 1992; Gould, 2001; Samore, 2021; Zilinskas, 1997).

Historically there have been several events involving intentional release of biological agents, e.g. the 1978 ricin poisoning of Georgi Markov in the U.K (Papaloucas et al., 2008), the *Salmonella* release in Oregon, U.S in 1984 (Thomas et al., 1997) and the anthrax letter attacks in the U.S. in 2001 (Jernigan et al., 2002). During World Wars I and II, biological warfare was planned and used by multiple countries with increasing capability and potential following technological developments. During the cold war, biological warfare was part of the defence research programmes of many countries, including the Soviet Union and the United States (Frischknecht, 2003). More recently, there have also been events and foiled attempts, which continue to highlight the threat. Deliberate releases of biological agents include the thwarted ricin bomb plot in Cologne, Germany in 2018; and the numerous parcels sent to consulates and embassies in Australia in 2019 ("CNN report on suspicious packages," 2019; "The Guardian article on suspicious packages," 2019; "NBC report on Ricin in Germany," 2018).

Novel Threats

Several biological agents (bacteria, viruses and toxins) can be considered as threats due to the relative ease of production and known technological possibilities for weaponization (CDC, 2023; Franconi et al., 2018). Traditional agents associated with the highest bioterror risk, are naturally occurring and cause serious animal and/or human infections or intoxications. The US Centers for Disease Control and Prevention (CDC) has classified biological agents into different categories, based on the risk they pose to national security (CDC, 2018). Agents predicted to cause high mortality rates and have the potential for major public health impact are placed in the highest risk category. Most of these high-risk agents, such as *Bacillus anthracis*, *Yersinia pestis* and *Francisella tularensis* are zoonotic, being able to infect both animals and humans. However, priority agents also include naturally occurring toxins such as *Clostridium botulinum* toxin and ricin (from castor beans) as well as emerging pathogens that could be engineered for mass dissemination in the future.

The consequences of biological threats can be equally serious regardless of whether a disease is naturally occurring, accidental (due to laboratory accidents, ignorance or negligence) or intentional

(sabotage, bioterror or warfare) (Franconi et al., 2018). The COVID-19 pandemic demonstrated the enormous global effect a pandemic can have. The origin of the virus is debated, and we will probably never be certain if the pandemic had a natural origin, was the result of a laboratory escape or even, more controversially, the result of an engineered virus escaping from a research institute (Borsetti et al., 2022; Frutos et al., 2022). The rapidly increasing number of high containment laboratories worldwide may increase the risk of deliberate or accidental release and poses biosecurity concerns, particularly when coupled with weak oversight of dual use research areas (Koblentz, 2023).

Recent advancements in synthetic biology have increased concerns regarding the potential for non-state actors worldwide to either obtain or construct and weaponize biological agents. The heightened risk of non-state actors acquiring such agents is further exacerbated by emerging trends such as 'DIY' biology and DNA synthesis, significantly expanding the capacity for non-state actors to manufacture agents in unregulated settings (Trump et al., 2021). Furthermore, there's a recent resurgence in concerns about state actors potentially planning to utilize bioweapons. This may involve actions carried out through proxy actors or hybrid warfare strategies, creating 'grey zone' challenges, where conflicts hover ambiguously between war and peace, making it difficult for traditional civilian stakeholders to effectively respond to hybrid attacks (Granholm et al., 2023). Hybrid warfare shares striking similarities with terrorism, involving deliberate attacks on civilians, medical responders, and healthcare facilities (Granholm et al., 2023).

An understanding of recent advances in technologies that could contribute to bioweapon development is crucial for preparedness and response planning, particularly in the context of terrorism. It is challenging to grasp the rapidly evolving landscape of technologies that can create or enhance organisms, increase harmful toxin abundance, or enable the rapid dissemination of dangerous adaptations. Given the need for cross-sectoral responses, this information must be condensed into a format that provides a snapshot for multiple sectors. Staying abreast of these technologies is essential for developing effective detection and surveillance mechanisms, and insights into policy gaps are vital for informing regulatory frameworks. The identification and assessment of potential threats, vulnerabilities, and risks associated with biotechnological advancements are integral components of this understanding.

Reviewing the current knowledge of genetic engineering, protein expression, and whole genome sequencing is not only crucial for identifying and assessing potential risks but also for guiding the

development of countermeasures, specialized training, and international collaboration. This comprehensive approach contributes to proactive mitigation of biosecurity threats and ensures preparedness for potential bioterrorism incidents.

The Dual-Use Potential of Synthetic Biology

The term 'synthetic biology' generally refers to man-made biological agents and encompasses the concepts, approaches, and tools employed to modify existing living organisms or create new ones. This scientific field employs engineering-based modelling and construction techniques (Trump et al., 2021) and is ubiquitous in the modern world, underpinning many biological applications. Techniques and methods were developed for beneficial purposes such as the development of new vaccines and medicines, to improve agricultural yields and for remediation of pollution. However, technological advancements can also be repurposed for malicious intentions and may be easily accessible to individuals with relevant knowledge and access to adequate facilities (National Academies of Sciences & Medicine, 2018). This kind of research, which holds potential for harmful misuse, is termed Dual-Use Research of Concern (DURC) (Rath et al., 2014). Various related terms are utilized to emphasize the dual-use potential of pathogen research, such as gain-of-function (GOF) research, gain-of-function research of concern (GOFROC), or enhanced potential pandemic pathogen (ePPP) research. Discussions on policy considerations and regulations pertaining to these aspects of research have recently been undertaken (Schuerger, 2023).

Synthetic biology involves techniques and technologies that can be used to synthesize novel DNA/RNA or edit the genome of existing known organisms to make them more harmful. The complexity of biological life implies that there are many barriers and steps that need to be overcome in order to "succeed" in modifying or creating new living organisms with pathogenic potential. However, rapid developments within this field, such as the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein) gene editing techniques and the improvements of DNA synthesis and assembly methods, have caused growing concerns about the misuse potential (Hughes & Ellington, 2017; Sun et al., 2022). Examples that illustrate important milestones within the field are well described in literature. In 2002, researchers constructed a synthetic poliovirus, demonstrating for the first time the ability to use genetic engineering to create an infectious agent solely by following instructions from a written sequence (Cello et al., 2002). When

researchers in 2016 constructed a synthetic horsepox virus (Noyce et al., 2018), this showed that also larger and more complex viruses could be generated based on publicly available sequences, and the researchers stated that this demonstrated that no viral pathogen is beyond the reach of synthetic biology (Noyce et al., 2018). The publication led to an international debate regarding ethical considerations in publishing this kind of information for open access and the dual-use risk of misuse, in this specific case for recreating smallpox virus (DiEuliis et al., 2017; Koblentz, 2017). Other examples where synthetic biology, for beneficial purposes, has been used to recreate or create infectious viruses with high consequence disease potential are the synthesis of a vaccine resistant mousepox virus in 2001 (Jackson et al., 2001), the reconstruction of the Spanish influenza virus in 2005 (Tumpey et al., 2005), and the synthesis of an outbreak strain of Ebola virus in 2019 (McMullan et al., 2019).

Combining synthetic biology with machine learning and artificial intelligence (AI), additionally increases the possibilities for dual-use issues. The accelerating development of AI is changing the global biosecurity risk assessment, and there are rising concerns that established biosecurity measures will not be sufficient to prevent deliberate or accidental malicious use of synthetic biology (Trump et al., 2020).

Aim

This review examines the rapidly advancing field of synthetic biology, recognizing its potential for misuse, particularly in the context of potential bioterror attacks or biological warfare. Our primary goal is to evaluate and articulate the current state of knowledge regarding the potential misuse of synthetic biology. Due to the expansive nature of this subject, our review focuses on synthesizing existing literature to comprehensively map and describe the knowledge pertaining to novel technologies that could be exploited for: 1) re-creating known pathogenic viruses and bacteria, 2) enhancing the pathogenicity of existing bacteria and viruses, and 3) synthesizing harmful biochemicals through in situ processes. Additionally, we also explore the role of whole genome sequencing and the open access data it produces that underscore many of the technologies examined in this review.

This targeted approach aims to provide an understanding of the misuse potential of synthetic biology, thereby contributing valuable insights for informed risk assessments, decision-making and strategic planning in the realm of biosecurity.

To facilitate cross-sectoral responses, we aim to distil this information into a concise format, offering a snapshot relevant to multiple sectors. Staying informed about these technologies is crucial for identifying and assessing potential risks and vulnerabilities and developing robust detection and surveillance mechanisms. Additionally, insights into policy gaps are indispensable for shaping regulatory frameworks, forming a foundation for global coordination in preventing, preparing, and responding to the malicious use of biotechnological advancements.

Methods

Search strategy

A comprehensive literature search was conducted in the period December 2021 to February 2022, targeting literature published between January 2016 and February 2022 using PubMed, Web of Science, Scopus, and Google Scholar. The search covered five themes: Recombinant protein/ mRNA/ gene expression; CRISPR; WGS; Metabolic engineering; and Synthetic biology. Search terms are detailed in table 1. The systematic search was complemented with manual searches to capture additional literature and data from relevant "grey literature" such as websites, reports, or protocols based on authors' prior knowledge and experience. Reference lists of papers from the included articles were also screened for relevant articles.

The following search terms were applied:

"Recombinant protein expression" AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
"Recombinant protein expression" AND (dual-use OR "dual use")
"Recombinant protein expression" AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
"mRNA expression" AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
"mRNA expression" AND (dual-use OR "dual use")
"mRNA expression" AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
"Recombinant gene expression" AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
"Recombinant gene expression" AND (dual-use OR "dual use")

"Recombinant gene expression" AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
CRISPR AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
CRISPR AND (dual-use OR "dual use")
CRISPR AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
(WGS OR "whole genome sequencing" OR metagenomics) AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
(WGS OR "whole genome sequencing" OR metagenomics) AND (dual-use OR "dual use")
(WGS OR "whole genome sequencing" OR metagenomics) AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
"metabolic engineering" AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
"metabolic engineering" AND (dual-use OR "dual use")
"metabolic engineering" AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
"synthetic biology" AND ("biosecurity risk" OR bioterrorism OR bio-terrorism OR bioweapon OR bio-weapon OR biothreat OR bio-threat)
"synthetic biology" AND (dual-use OR "dual use")
"synthetic biology" AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")
"synthetic biology" AND ("biological toxins" OR bio-agent* OR bioagent* OR "biological agent" OR "biological warfare agents")
(bioterrorism OR bio-terrorism OR biosecurity OR bio-security OR biocrime OR bio-crime OR bioweapon OR bio-weapon OR biothreat OR bio-threat) AND (DIY OR "do it yourself" OR do-it-yourself OR garage OR "garage biology")

Table 1. Search terms used to interrogate PubMed; Web of Science, Scopus and Google Scholar Inclusion dates from December 2016 to February 2022.

Inclusion criteria

Literature was included if it met the following criteria: 1) Described data relating to one or more of the following actions: i) "re-creating known pathogenic viruses and bacteria" or ii) "making bacteria or viruses more pathogenic" or iii) making harmful biochemicals via in situ synthesis; 2) Described data relating to the production of synthetic pathogens irrespective of the method of data collection or instrument used or agent in play, 3) Pertained to 'dual-use' technologies, i.e. those technologies

developed for altruistic purposes that have the potential for nefarious manipulation 4) Provided relevant background information or discussing risks, 5) Commented on ethical considerations surrounding the development of dual-use technologies 6) Proposed solutions or recommendations and 7) Were published between January 2016 and February 2022. Papers published before January 2016 and after February 2022 were included on a case-by-case basis if deemed relevant. Additional literature was included on a case-by-case basis.

Exclusion criteria

The following exclusion criteria were applied: 1) Duplicated data, 2) Not in English or language of Joint Action TERROR partner, 3) Outside the scope of the review, 4) No full text available and 5) Other reasons, including papers not relevant to bioterrorism.

Literature assessment

We aimed for a transparent and systematic process of assessing the literature, whilst also ensuring a pragmatic approach in the light of available resources of time and funding.

The search results were distributed within the project group and the records reviewed in parallel by teams of 2-4 experts per topic to assess whether they fulfilled the inclusion or exclusion criteria. Each team focussed on a different topic as used in the search strategy:

- Recombinant protein/ mRNA/ gene expression
- CRISPR
- Whole Genome Sequencing
- Metabolic engineering
- Synthetic biology

The first sift of papers was conducted to remove obvious excluded papers. A second sift was conducted using additional information from abstracts. Full texts were read on all papers that had passed through both sifts and narrative summaries were developed for each topic. Basic information about all included texts were recorded and Endnote (EndNote, 2013) was used to share a library of included text with all authors.

Results

Search results

The selection of relevant papers is depicted in a PRISMA flow diagram (Figure 1). In summary, 1502 papers were screened and 38 of these were deemed pertinent to the review and met the inclusion criteria. Additional material was included from organisational websites such as NATO, WHO, etc (n = 17); news articles (n = 5); papers published after initial search complete (n = 9); and papers selected by authors either through signposting from reference lists or to exemplify a discussion point (n = 32). Most of the 102 references (85%) were published after 2016.

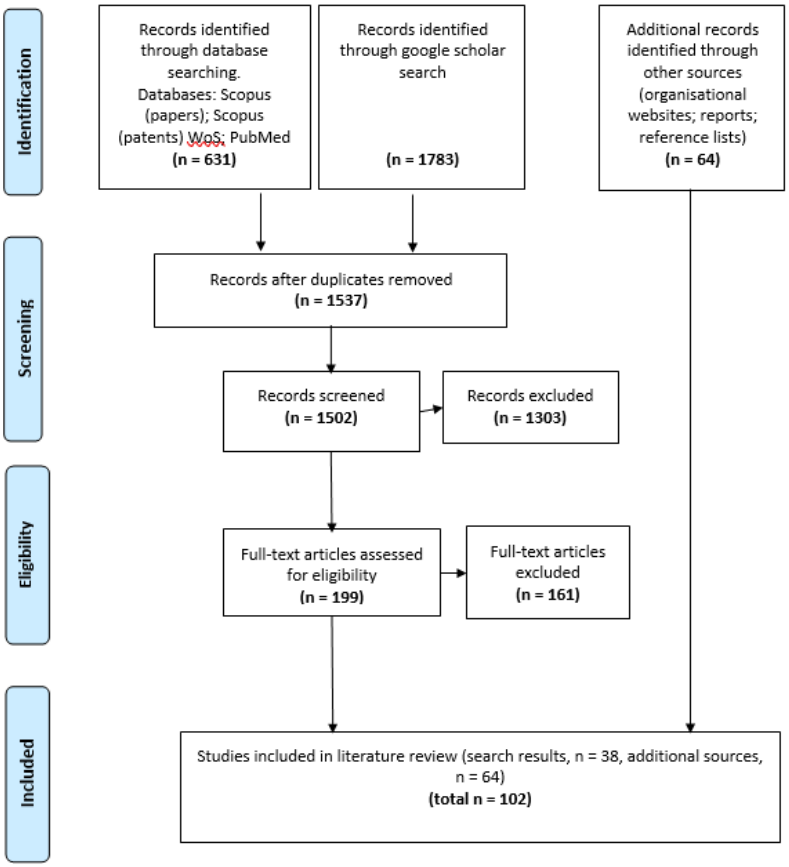

Figure 1. Flowchart to describe results of literature search

Genetic engineering

Technological overview

Genetic engineering manipulates the genomic composition of an organism by introducing, deleting, or modifying specific genes. There are a variety of ways that this might be done including through traditional recombinant and transgenic technologies where different DNA sources are combined, cloning to produce multiple copies of a gene of interest, and modern genome editing technologies to produce precise modifications within an organisms' genome.

Genetic engineering methods have been utilized by researchers for decades and span various fields, including agriculture, medicine and industry. Examples include the development of genetically modified crops with improved resistance to pests or environmental conditions, the production of pharmaceuticals using genetically modified microorganisms, and the treatment of genetic disorders through gene therapy.

Notably, the CRISPR/Cas system (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 systems) has revolutionized genetic manipulation possibilities making gene editing more accessible and efficient (VKM, 2022). Another easily accessible gene editing technique is TALEN (Transcription Activator-Like Effector Nucleases), a flexible technique that can specifically target any DNA sequence or DNA-associated protein, enabling editing of the genome, epigenome and transcriptome (Nemudryi et al., 2014) ((Becker & Boch, 2021). In addition to these techniques, developments within synthetic biology include Gibson Assembly technology and DNA synthesis, which encompass desktop DNA synthesizers capable of synthesizing long DNA fragments used as building blocks for synthetic genomes (Kobokovich et al., 2019).

While genetic engineering holds great promise for advancements in science and technology, it also raises ethical, environmental, and safety concerns. The deliberate release of genetically modified organisms into the environment and the potential for unintended consequences are areas of ongoing debate and regulatory scrutiny.

Technological advancements with dual-use potential

The potential dual-use of these novel gene-editing tools has raised concerns regarding the possible production of biological weapons and biothreat agents (Dieuliis & Giordano, 2017; Kobokovich et al., 2019; Wang & Zhang, 2019). Potential areas of misuse include editing microorganisms to increase their pathogenicity by adding toxins, virulence factors and/or genes associated with antibiotic/ drug resistance. Further concerns have been raised surrounding the modification of zoonotic agents to increase their transmissibility to humans or by creating novel infectious agents with unknown disease potential (Carter & Warner, 2018; Cummings et al., 2021; Holm, 2017; Walsh, 2018).

A transformational genome assembly approach was developed to engineer viruses with large DNA genomes rapidly and efficiently (Oldfield et al., 2017). This modular yeast-based approach was inspired by the technology developed to clone large regions of the human genome using homologous recombination in *Saccharomyces cerevisiae*. As a proof-of-concept, this approach, allowing the introduction of CRISPR modifications, was successfully applied on the herpes simplex virus type 1 (HSV-1). However, such an approach, with the ability to perform genome-wide editing through assembly methods of large DNA virus genomes, raised dual-use concerns. For example, it may be used to recreate genomes of pathogenic viruses, such as pox viruses from synthetic DNA. It may also be used to increase the virulence, transmissibility, or resistance to therapeutic applications against pathogenic viruses (Oldfield et al., 2017).

Genetic modifications and optimizations of *Yersinia pestis* using CRISPR technology were recently demonstrated (Ansari et al., 2020; Wang & Zhang, 2019). Using this technology, the production of multidrug resistant strains is possible, representing a crucial health risk because the use of antibiotics is the primary treatment for *Y. pestis* infection. In addition, there is a risk that such genome engineering may be used to generate strains with genome alterations that bypass standard detection methods or interfere with the protective effect of vaccines (Ansari et al., 2020).

The risk of using genetic engineering tools such as TALEN and CRISPR to increase the virulence of viruses was also discussed by D'Souza and Patel 2020 (D'Souza & Patel, 2020). The paper reviews the biosecurity risk categorization of Hantaviruses and raises awareness about the possibilities of misusing gene editing tools to create novel variants of the Hantavirus, with properties that enhance the virus' bioweapon potential.

Several examples of misuse potential in relation to specific pathogens are highlighted in the literature, such as the synthesis of poliovirus, Spanish influenza, Ebola, and vaccine resistant mousepox (Drew & Mueller-Doblies, 2017; Holm, 2017; MacIntyre, 2020; Melin, 2021). The synthesis of horsepox virus as demonstrated by Noyce et al (Noyce et al., 2018), especially raised concerns and sparked an international debate on the increased risk of terrorist groups recreating smallpox (Impelluso & Lentzos, 2017; Koblentz, 2017, 2018; Kupferschmidt, 2017).

Along with rapid advancements in genetic engineering, a trend known as Do-It-Yourself (DIY) biology has emerged, encouraging non-experts to conduct biological experiments even outside of professional laboratory settings. DIY labs have significantly increased the accessibility of life sciences to bio-enthusiasts, predominantly in major cities in Europe and the USA (Meyer & Vergnaud, 2020). These inexpensive, semi-professional laboratories can operate independently of traditional research environments. The emergence of low-cost 3D printing technologies and desktop DNA synthesizers has further democratized access to essential equipment beyond the confines of conventional institutional setups (Keulartz & van den Belt, 2016; Pilizota & Yang, 2018). There is a concern that the availability of such laboratories could be exploited by individuals or groups who wish to cause intentional harm (Sarpong et al., 2020). However, it has been claimed that the link between bioterrorism and DIY biology and the level of sophistication of the experiments typically performed in such labs might be overstated. Many members of DIY labs are biologists and most labs in the US collaborate with the FBI on security (Sarpong et al., 2020).

The risk of misuse increases as the number of companies offering easy-to-use gene editing kits at affordable prices, grows. Including CRISPR–Cas9 reagents in ready-to-use kits makes the technology more accessible to amateur molecular biologists (Smalley, 2018). An example of such a risk, although not developed for bioterrorism purposes, was the sale of a CRISPR bacterial gene editing Odin kit including a non-pathogenic *Escherichia coli* bacterial strain, a template DNA and plasmids containing Cas9, racrRNA and crRNA. This easily enables the generation of a K43T mutation to the *E. coli*'s *rpsL* gene, resulting in a streptomycin resistant *E. coli* strain. Its commercialization was banned in Germany following the contamination of the kit with pathogenic bacteria, although the risk of infection and spreading of AMR was assessed as low/very low by the European Centre for Disease Prevention and Control (ECDC, 2017; Smalley, 2018; Sneed, 2017). However, the risk of misuse through bioterrorism was not specifically addressed.

In summary, several articles were found that addressed the misuse-potential of novel genetic engineering tools, such as CRISPR. Increased accessibility of technology and knowledge, lower costs and increased simplicity of use are factors that could escalate future risks.

Heterologous expression and mRNA technology

Technological overview

Heterologous expression refers to the process of introducing and expressing a gene or genetic material from one organism into a different host organism. This process is commonly used in molecular biology and biotechnology to produce proteins, enzymes, or other molecules of interest that may be challenging to obtain directly from the host organism. By utilizing a different host with well-established expression systems, researchers can take advantage of the host's machinery to produce the desired protein. This is especially valuable in the production of therapeutic proteins, industrial enzymes, and other biotechnological applications.

mRNA (messenger RNA) technology involves the use of synthetic or modified messenger RNA to instruct cells to produce specific proteins. This technology has gained significant attention, especially in the context of vaccine development and gene therapy. Viral mRNA vaccines work by introducing a small piece of mRNA that codes for a viral antigen into the body. Once inside cells, the mRNA is translated into the viral protein, stimulating an immune response without using live or inactivated viruses. This approach allows for the rapid development of vaccines, as the mRNA sequence can be synthesized based on genetic information of the target pathogen.

These technologies have undergone significant refinement over the years, leading to their establishment as industrial standards. Proteins and peptides (such as toxins) that are naturally found at low abundance in rare organisms can today, in favourable cases, be produced in large quantities using rudimentary knowledge and infrastructure. This technique has gained prominence due to its ability to provide high-quality and pure protein samples for various disciplines, including biochemistry and structural biology. Noteworthy advancements include the development of engineered strains capable of expressing complex proteins and the use of gene design and gene synthesis for efficient cloning and expression (Baeshen et al., 2015). Design of proteins with new functions are facilitated by the ongoing deep learning revolution in 3D structure prediction. While these advancements offer

numerous opportunities in the fields of medicine, research, and biotechnology, they also present certain risks that need to be acknowledged and addressed.

Technological advancements with dual-use potential

The democratization of techniques used in heterologous gene expression makes them accessible to non-experts and necessitates increased awareness of the potential of misuse. Nefariously designed and produced effector proteins (e.g., toxins, bioregulators, prions, superantigens) ex vivo (protein) or in vivo (mRNA), in combination with effective administration systems, may constitute novel threats. Responsible use and risk mitigation strategies must be implemented to ensure the safe and ethical deployment of these technologies.

Several factors have contributed to the progress in heterologous protein expression including the availability of sequence databases (see section on WGS), advances in expression vector design, genetic construction, and choice of host (Goldenzweig et al., 2016; Norn et al., 2021; Weinstein et al., 2021). Ongoing efforts to optimize protein stability and heterologous expression levels, could result in the bacterial production of challenging targets, time and/or cost-effective alternatives to traditional cloning methods and the development of advanced expression systems capable of many types of post-translational modifications (Baeshen et al., 2015; Nothaft & Szymanski, 2019). Additionally, advancements in hardware and parallel processing allow hundreds or even thousands of permutations to be rapidly evaluated in order to identify the optimal combination. In such optimization efforts, a several orders of magnitude increase in production yield may be achieved.

In summary, the recent advancements in heterologous protein expression and mRNA technology have opened up new possibilities in the fields of medicine, research, and biotechnology. These techniques offer improved efficiency, flexibility, and production yields, paving the way for transformative breakthroughs. However, it is essential to consider the associated risks and ensure responsible use to mitigate any unintended harm.

Whole genome sequencing (WGS)

Technological overview

Whole genome sequencing (WGS) is used to characterise the DNA or RNA sequence of an organism and is a fundamental technique in biological research. It has proven indispensable for understanding the diversity of pathogens and identifying genes associated with virulence, antibiotic resistance, transmissibility, and host-pathogen interactions. This genomic characterization not only aids in the precise identification of infectious agents but also forms the basis for a multitude of applications in the field of biological research.

Having been used in research for decades, WGS can no longer be described as novel. However, in recent years, next generation sequencing (NGS) technologies have undergone significant advancements, resulting in heightened sequencing speed, accuracy, and cost-effectiveness. Key trends include the emergence of third-generation sequencing technologies like nanopore sequencing, which offer long-read capabilities, enabling more accurate assembly of complex genomes and improved detection of structural variations. Additionally, the huge amount of genomic data that now exists coupled with computational advances in areas such as artificial intelligence (AI) and bioinformatics have facilitated the analysis and interpretation of large-scale genomic datasets. Machine learning approaches are increasingly being applied for variant calling, annotation, classification and prediction.

The wealth of genomic data made available through WGS is often deposited into publicly accessible databases, facilitating scientific research and exploration. Prominent examples include the European Bioinformatics Institute's European Nucleotide Archive (ENA) and the National Center for Biotechnology Information's GenBank. These databases store genomic sequences, annotations, and related information, providing a comprehensive resource for researchers worldwide. Access to these databases is generally open to the public, allowing scientists, educators, and even the broader community to explore genomic data. The ease of access varies; while basic searches and retrieval are straightforward, advanced analyses may require bioinformatics expertise. Researchers can access a wide array of data, including DNA sequences, gene annotations, and information on genetic variations. The availability of such databases promotes collaboration, accelerates scientific

discoveries, and underlines the importance of responsible data sharing within the scientific community.

Dual-use considerations of whole genome sequencing

WGS techniques is particularly valuable in detecting, identifying, source tracing (e.g. outbreak and bioforensic investigations) and monitoring engineered biothreat agents by scrutinizing unusual patterns of virulence genes, antimicrobial resistance (AMR) genes , and other indicators of engineered genetic alterations (Keshava et al., 2018; Massey, 2016; Minogue et al., 2019; Nwadiugwu & Monteiro, 2022; Oliveira et al., 2020). Although many studies discuss the advantages and disadvantages of using WGS for the characterization of genetically engineered bioweapons, very few case studies exist in the literature and remain mostly limited to cases where naturally occurring pathogens were used as bioweapons (González et al., 2017). For instance, WGS helped to confirm that the anthrax associated to letters in the USA in 2001 constituted a deliberate bioterrorist attack. Genomic analysis confirmed that the anthrax letter strain corresponded to the Ames strain, a rare type isolated in 1981 from a dead cow in Texas and subsequently sent to the U.S. Army Medical Research Institute of Infectious Diseases for vaccine investigation purposes. Based on WGS, it was demonstrated that letter attack strain contained four specific mutations shared with a unique source batch, eventually allowing tracing the attack strain back to a specific U.S. Army facility (Rasko et al., 2011).

Described case studies where WGS was used during an outbreak to characterize a biological agent specifically engineered to become more dangerous remain absent (beyond the introduction of specific AMR genes). In this regard, it may be relevant to consider the field of genetically modified (micro-)organisms in agricultural/food/feed applications, for which some case studies already exist demonstrating how genomics can be used to characterize unnatural modifications in micro-organisms, and therefore can be used as a framework on how WGS could be implemented for biothreats (Minogue et al., 2019; Nwadiugwu & Monteiro, 2022).

Numerous studies have emphasized the beneficial aspects of WGS in responding to bioterrorism, however, sequence-based genomics has enabled databases populated with genomes of highly virulent pathogens, including those with AMR and toxin-encoding genes to be publicly available. While these databases are crucial for research, diagnostics and forensics, the sharing of detailed information furnishes comprehensive insights into pathogens, encompassing details about their origin, transmission routes, virulence factors (e.g., toxins), and AMR (Gargis et al., 2019; Kambouris

et al., 2018; Oliveira et al., 2020) which could potentially be misused to create dangerous pathogenic bioweapons (Nwadiugwu & Monteiro, 2022; Vinatzer et al., 2019). A review by Vinatzer et al (Vinatzer et al., 2019), highlights the risk that these databases may become targets of cyber-bio-attacks interfering with public health and biosecurity systems.

In summary, the examined literature highlights the risks linked to publicly available genomic databases and the potential misuse of sensitive genetic information. However, WGS plays a vital role in identifying and characterizing anomalies or deviations from established genetic patterns in outbreak strains. This technology is crucial for distinguishing between natural occurrences and intentional dissemination of agent(s). Additionally, genomics is central to attribution, aiding in identifying the actor responsible for any illegitimate activity. To enhance rapid response and accurate characterization of suspicious biothreat events, ongoing research and development are essential to tackle the limitations and challenges associated with applying genomics effectively.

Summary of identified risks of technological advancements within Synthetic Biology

From the included studies, several of the examined molecular techniques were found to be associated with a misuse-potential. Table 1 summarizes risks associated with the techniques that were assessed in the literature review. Importantly, as stated earlier, the technological developments described in the results section are just examples that were identified in the included literature and not an exhaustive list, as making a complete list on developments within synthetic biology is beyond the scope of this report.

Table 1: Overview of potential risks posed by selected molecular techniques

Technique	Action	Risk	Reference(s)
Genetic engineering (synthetic)	Modification of existing pathogens/ reconstruction of virulent strains	Synthetic biology techniques can be used to modify existing pathogens by introducing specific genetic changes. This could involve enhancing their virulence, altering their host range, increasing their transmissibility, or making them resistant to existing treatments, including vaccines and antibiotics. Also, pathogens can be modified so that they are not detectable by standard diagnostics or create hoax for malicious deception (create false positives in detection systems).	(Carter & Warner, 2018; Cummings et al., 2021; Dieuliis & Giordano, 2017; Holm, 2017; Imperiale et al., 2018; Kobokovich et al., 2019; Walsh, 2018; Wang & Zhang, 2019)
	Creation of novel pathogens (de novo synthesis)	Synthetic biology techniques, such as gene synthesis and genome editing (e.g., CRISPR/Cas), allow for the creation of novel pathogens. Bioterrorists could potentially design and engineer entirely new infectious agents that are highly virulent, resistant to treatment and/or transmissible to and between humans. Also, novel pathogens can be created so that they are not detectable by standard diagnostics.	(Cello et al., 2002; DiEuliis et al., 2017; Koblentz, 2017, 2018; Noyce & Evans, 2018; Noyce et al., 2018; Tumpey et al., 2005)

Heterologous protein expression and mRNA technology	Producing harmful effector macromolecules in biological systems	Expression of effector macromolecules, such as proteins or other biomolecules, using heterologous expression systems can be exploited to create harmful biological agents. In the wrong hands, these technologies could be used to produce toxins or other dangerous substances with the intent to cause harm. The dual-use nature of these techniques means that the same processes and knowledge used for legitimate purposes can be repurposed for malicious intent. The ability to engineer proteins or modify RNA sequences could make it harder to detect and attribute the source of a bioterror attack	(Goldenzweig et al., 2016; Norn et al., 2021; Weinstein et al., 2021)
	Delivery systems for within host production of harmful effector macromolecules	mRNA technology, in particular, offers advantages in terms of faster production and flexibility. This could potentially facilitate the rapid production and dissemination of harmful biological agents on a larger scale, amplifying their impact and making them harder to contain.	(Lokugamage et al., 2021; Patel et al., 2019; Qiu et al., 2019; Zhang et al., 2020)
Whole genome sequencing (WGS	Elucidating and sharing detailed microbial sequence information through sequence databases or in open science.	WGS enables the identification and characterization of pathogenic traits, such as virulence factors or antibiotic resistance genes, in microorganisms. This information can be potentially misused by bioterrorists to engineer or enhance the pathogenicity of existing pathogens. Research and sequence information published in open access databases can be used to design and synthesize specific DNA/ RNA sequences. Bioterrorists could exploit this technology to create synthetic DNA/ RNA fragments or even entire genomes of dangerous pathogens, bypassing the need to obtain the actual biological samples. This increases the risk of developing novel infectious agents or resurrecting extinct pathogens.	(Nwadiugwu & Monteiro, 2022; Smith & Sandbrink, 2022; Vinatzer et al., 2019)

Discussion

Synthetic biology is a developing discipline of huge potential. Progress in areas such as genetic engineering, heterologous gene expression and mRNA technology is coupled with an increased ease of access to accurate genomic data and ushered in transformative possibilities in various fields.

While these technologies offer substantial benefits for medicine, research, and biotechnology, they concurrently present challenges and risks, particularly in the context of biowarfare and bioterrorism.

While we found no examples in the examined literature of synthetic biology being accidentally or deliberately used to cause harm, we did find several examples of synthetic biology being used to recreate or modify highly pathogenic agents for beneficial or research purposes. This observation, combined with the accelerated technological developments within this field, including the availability of open science and sequence databases, raises concerns about the potential for misuse.

Here we discuss potential risk assessment and mitigation strategies applicable to bioterror and biological warfare aspects of synthetic biology considering the identified literature.

Risk assessment strategies in the field of synthetic biology

Different strategies can be used to assess risk. In its simplest sense, the magnitude of risk is commonly considered as the product of the probability and consequence of an adverse outcome, which involves a certain degree of uncertainty. In this review, we have focused on risks associated with synthetic biology and how it pertains to 1) the re-creation of known pathogenic viruses and bacteria, 2) the making of existing bacteria and viruses more pathogenic or 3) the making of harmful substances in biological systems. Additionally, we have explored the role of whole genome sequencing and the open access data it generates that underscore many of the technologies examined in this review.

Based on current knowledge and developments, we discuss our findings in light of aspects that should be considered when assessing the risks associated with synthetic biology in the context of the three areas above, as well as strategies that could mitigate identified risks.

Availability, cost, and simplicity of technologies

Democratization, simplicity, increased precision and availability of gene editing and sequencing technologies are important aspects that increase biosecurity risks (Ahteensuu, 2017). Traditionally, the synthesis of longer sequences (>200 bp) has been considered too expensive and complex to be

performed by non-professionals. However, the development towards the commercialization of DNA synthesis (e.g. desk-top DNA synthesisers) and assembly methods, lowers the threshold for potential misuse (Hoose et al., 2023). In addition, the field of heterologous expression has been developing gradually but constantly. Now, these techniques are more easily accessible, more efficient, simpler, cheaper, and more democratized than ever, having become routine in almost any research or biotech/pharmaceutical laboratory. Even advanced capabilities in gene editing technologies and heterologous protein expression are accessible to non-experts, and undergraduate students can successfully undertake relatively advanced constructs for the successful production of various functional proteins. Example of this include the yearly synthetic biology competition iGEM, which in 2023 included 400 multidisciplinary teams from more than 45 countries and regions (*iGEM competition*).

Communication, dissemination, and ethical aspects

Information on biotechnology has never been more accessible to individuals. High-speed internet is available all over the world, and everyone can find numerous examples on-line or in popular literature, on how synthetic biology could be used by a malevolent actor (Nieuwenweg et al., 2021). Artificial intelligence tools available for the general public increase the access to information and especially the ability to rapidly combine relevant information (Helena, 2023). To complicate the picture, some actors intentionally spread misinformation. The vast amount of available information makes it difficult to distinguish "real news" from "fake news" and is therefore challenging to evaluate the true risk of synthetic biology. Some actors may have reasons to downplay this risk in the public debate while others might have reasons to exaggerate it (Melin, 2021).

Information sharing on advances in synthetic biology thus raises a range of ethical issues, and the risk of unintended consequences need to be addressed. Widely publicising the methodology by which a person or a laboratory can assemble and synthesize high consequence pathogens is an example of an information hazard (Esvelt, 2018). The research community has a natural drive towards and responsibility of sharing their knowledge, methodology and findings (including sequences) with the international scientific community in public journals, repositories or elsewhere. This sharing of "know-how" might inspire terrorists by demonstrating *what* is technically possible and *how* to do it (Ahteensuu, 2017). One publication especially causing international debate was the publication of the detailed methodology of the *de novo* synthesis of horsepox virus (Koblentz, 2017). Such concerns

were actualized more recently during the COVID-19 pandemic, with extensive dissemination of detailed scientific knowledge on the genotypic and phenotypic characteristics of the pathogen. The availability of such information increases the risk of deliberate genetic modification and release of , for example novel SARS-CoV-2 variants of concern (Musunuri et al., 2021).

Researchers, scientific journals, and reviewers all have an ethical responsibility to assess if there is biosecurity risk associated with a potential publication, but there are no international harmonized guidelines or regulations stating how to approach this dilemma. Non-reviewed scientific work is more frequently published on-line as "preprints", an option that was much used during the COVID-19 pandemic, to share novel information in a rapid and accessible way. Although preprints accelerate dissemination of findings and might contribute positively to our ability to deal with emerging biological threats, increased use could also imply a biosecurity risk, as the control mechanisms in the publishing process provided by scientific journals is lacking. To address the risk with open science, a transparent process with external evaluation throughout the research process has been recommended, where funders and institutions also have an important role to play in improving biosecurity (Smith & Sandbrink, 2022).

Biological aspects

When assessing the misuse potential and risks associated with synthetic biology it is important not only to consider the technology itself but also the complexity of biological systems. One of the major challenges in synthetic biology is the lack of predictability and reliability of complex biological systems. This complexity might serve as a barrier against deliberate misuse since targeted modification of microbial genomes requires extensive knowledge, appropriate network and resources.

Another important aspect is the genetic stability of microorganisms (Arbel-Groissman et al., 2023). While impressive functionality has been achieved from engineering microorganisms grown in stable defined media, a major hurdle that remains is the issue of genetic stability in conditions with biological competition or chemical or physical variation in the ambient environment. Introduced genome alterations, adding functions such as secondary metabolites not needed for primary cell survival, are prone to mutation, recombination and functional loss and can quickly drive populations away from desired phenotypes. Though there are ways to circumvent this, it remains a considerable challenge

(Kumar & Hasty, 2023). Most synthetic constructs induce a fitness cost to the cell that is unstable in the face of prolonged selection pressure, and therefore, not all pathogenic or pathogenic-enhancing constructs made in a laboratory may constitute a risk in a bioterrorism or a biowarfare scenario. Thus, when developing future risk assessment frameworks, it will be important to take into account fitness and stability issues as well as the complexity of biological systems.

Actor, intent, and capability

The perspective of actor, intent, and capability is crucial when considering risks and threats associated with synthetic biology. This aspect, while outside the scope of our literature review, must consider a range of actors from state-sponsored entities to terrorist cells, insider threats, and individual "lone wolf" actors.

Risk mitigation strategies

Mitigating the risk of synthetic biology-mediated bioterror-attacks could be approached by i) reducing the *probability* of an attack and ii) minimizing the *consequences* of an attack (National Academies of Sciences & Medicine, 2018). However, while strict regulations of access to technology and knowledge could be effective in mitigating the probability of misuse, the same measures would likely hamper innovation and use for beneficial purposes. Thus, these conflicting considerations and needs, must be balanced in future strategies to enable sound and responsible developments.

The following provides examples of risk mitigation strategies, as identified in the examined literature.

Controlling the spread of "know- how" and improving awareness in research communities and industry

Biosecurity and biosafety measures, coupled with training initiatives, serve as effective tools to manage the risks associated with the dissemination of sensitive knowledge. However, the necessity for censoring information, including techniques, instruments, and biological components, challenges the fundamental scientific practice of openly sharing research findings. Disparities in awareness levels exist both among researchers and institutions, potentially leading to a lack of risk consideration in some organizations or an overly cautious approach that hinders valuable research. To address these challenges, targeted training initiatives, originating at the national and institute levels, are essential. Implementing a systematic risk-assessment approach empowers scientists to conscientiously incorporate risk considerations into their research endeavours.

Controlling access to technology and sensitive genomic data

To reduce risks related to publicly available sequence databases, recommendations have been made to restrict and regulate access to sensitive genomic data (Nwadiugwu & Monteiro, 2022; Schmedes & Budowle, 2019; Vinatzer et al., 2019). Furthermore, the International Gene Synthesis Consortium (IGSC) was created in 2009 to set protocols for the screening of ordered sequences and to check the credentials of those who place the order (*International Gene Synthesis Consortium*; Leo Elworth et al., 2020). Ordered sequences are checked against a range of databases that include; pathogenic microorganisms and genes (U.S. regulated pathogens (select agents), Australia Group (AG) list agents, U.S. Commerce Control List (CCL) controlled sequences, and European Union (EU) sequences. However, there are challenges connected to the current screening process for synthetic DNA. IGSC is industry-led and represents most of the commercial gene synthesis capacity worldwide. To our knowledge there are no international requirements for companies to follow these guidelines. In addition, desktop DNA and RNA assemblers and synthesizers for long nucleic acids are available, allowing sequences to be generated independent of commercial companies. However, these are considered as dual-use equipment by the Australia Group (AG) and listed on their control list (*The Australia Group Control Lists*). To mitigate the increased risk of misuse relative to democratization of CRISPR/Cas9 there has been a call for a much more concerted, international, public debate about monitoring and possibly regulating access to some of those tools (Imperiale et al., 2018).

Tools and competence to rapidly detect and identify synthetically engineered pathogens

The need for new tools and methods to rapidly identify unnatural outbreaks including the detection of gene edited organisms, has previously been highlighted (MacIntyre, 2020). As the number of genes targeted for modification is with endless possibilities, efforts must be prioritized to fully develop and broadly implement a methodology that is able to distinguish synthetic modifications from mutations and recombinations occurring by natural processes. This can be challenging and requires expertise of DNA/RNA sequencing and bioinformatics for the detection and identification of engineered agents. Due to the inherent complexity of genomics, purpose-oriented laboratory networks should be instigated that have a demonstrable proficiency in genomics (Plamboeck et al., 2016). An example is the Ref Bio project (*RefBio project - German Contribution to Strengthen the Reference Laboratories Bio in the UNSGM*) which is under the auspices of the UN Secretary General's Mechanism for Investigation

of Alleged Use of Chemical and Biological Weapons (UNODA, 2023). The Ref Bio project organizes each year proficiency tests to evaluate the capability of international laboratories to identify and characterize pathogens from datasets generated by modern DNA sequencing techniques, with the overall aim to increase the level of preparedness for laboratory participation in possible future UN investigations of alleged use of biological weapons and improve the methods for sequence data analysis for pathogen identification and characterization.

Surveillance, intelligence gathering and threat assessment

Experiences from the COVID-19 pandemic have revealed several areas of improvement in the surveillance of serious cross-border health threats. Several initiatives and actions have been initiated to strengthen and harmonize surveillance capabilities within the European Union (*EU4Health programme 2021-2027*; *Health Emergency Preparedness and Response (HERA)*; *United4Surveillance*) , taking into account the need for digitalization, integration and effective and secure transfer and sharing of data across countries and in the frame of the One Health approach. To ensure that surveillance capacities are designed to detect and handle the full range of possible biological (B)scenarios, both natural and intentional B-scenarios should be included to guide future surveillance strategies and developments. This would likely require a cross-sectoral approach where continuous intelligence gathering, threat assessments and horizon scanning are key components.

Therapeutics/vaccines

The COVID-19 pandemic demonstrated the need for preparedness for novel disease outbreaks and epidemics, regardless of the whether these could be naturally occurring, accidental or intentional. Rapid and effective response relied on years of foundational research in immunology and infectious disease, as well as advances in healthcare, diagnostics, and treatment. Advancements within synthetic biology paves the way for new and more efficient ways of designing and delivering targeted vaccines and therapeutics (Cubillos-Ruiz et al., 2021). For example, mRNA vaccines developed by Pfizer-BioNTech and Moderna were approved for emergency use within a year of the COVID-19 pandemic onset, a remarkable achievement that relied on years of basic research in the field. This underscores the need for ongoing investment in healthcare, diagnostics, and basic research to ensure that we are prepared for the next incident, whatever it may be. Engineered pathogens as well as novel "natural" health threats can potentially be equipped with novel or unusual combinations of traditional

virulence factors, making traditional therapeutics or vaccines less effective. Thus, supporting research initiatives, scientific advancements, and international collaborations within the field of medical countermeasures (MCMs) are important steps in strengthening national and international health preparedness against novel biological threats.

Limitations (methodological)

- There are many ongoing technological advancements in synthetic biology and making a complete list of developments is beyond the reach of this report, since prediction of the future of such a broad field is by definition predestined to be incomprehensive. Thus, the developments described are just examples and not a complete picture of the technological advancements in the field.
- Search results of the literature review are restricted by the search strategy, e.g., selection of search terms, the time frame of the search as well as the inherent limitations of only examining open-source literature.
- Artificial Intelligence (AI) has become a new and powerful tool as this review is being finalised. It is the opinion of the authors that this technology will accelerate both scientific advancements, but also increase the risk regarding synthetic biology in the future.

Conclusion

As the technological development within the field of synthetic biology rapidly evolves, a wealth of potential beneficial applications opens to different parts of the society, such as the health sector, energy sector, agricultural sector, research communities and private industries. However, with the rapid development and increased availability of synthetic biology follows increased risk of misuse.

In this literature review, being limited to open sources, we found no examples of synthetic biology being accidentally or deliberately used to cause harm. On the other hand, examples of synthetic biology being used to recreate or modify highly pathogenic agents for beneficial or research purposes,

clearly demonstrates what is technically possible. This observation, combined with the accelerated technological developments and availability, are causing concerns and calls for increased awareness and coordinated efforts to mitigate the risks.

A greater engagement with stakeholders, including the public, is needed to ensure that synthetic biology is developed and used in a safe, responsible and transparent manner.

Within academia and research, there is a need for greater education and workforce development to ensure that there is a skilled and diverse workforce to drive innovation in this field. Education programs should also include awareness raising on ethical and legal risks. This will require the development of new training programs, as well as efforts to increase diversity and inclusion in the field.

Raising awareness among scientists, stakeholders, and policymakers is crucial in minimizing the potential misuse of these technologies. By harnessing the opportunities and addressing the risks, the scientific community can continue to leverage these advancements for the betterment of society and contribute to a more sustainable and ethical future.

Improved risk assessments and the broader use of secure laboratories for experiments with potential dual-use implications should lead to improved biosecurity. The growing volume of research and the increasing number of researchers employing synthetic biology techniques inherently raises the likelihood of unforeseen incidents, whether stemming from lack of knowledge, accidents, inadequate planning, or negligence.

While the intention is not to cause harm, it's impossible to entirely eliminate this risk. However, it needs to be balanced against the positive outcomes and societal benefits of the research being pursued. One reassuring aspect is that most experiments conducted with good intentions are not designed to optimize the fitness of the microorganisms outside the laboratory environment. Biological competition and environmental factors to which the organisms are not adapted to, might reduce the consequences. However, if experiments are conducted with the purpose of causing harm and are carried out covertly, the risks increase significantly. It is practically impossible to detect intent, as the development or production of dangerous agents can be done on a small scale and decentralized, making it easy to conceal.

Given the widespread availability of knowledge and technology in the field, it is essential to be prepared for the possibility of an incident by having international cooperation and governance

frameworks, well-developed healthcare systems, efficient diagnostic tools, and a strong foundation of international collaboration and basic research in relevant areas. By building a strong foundation of knowledge and capabilities, we can minimize the impact of incidents and help to ensure the health and safety of individuals and communities around the world, while also continuing to improve biosecurity and biosafety to prevent incidents from occurring in the first place.

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