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Review of science and technology developments relevant to the Convention, including for the enhanced implementation of all articles of the Convention as well as the identification of potential benefits and risks of new science and technology developments relevant to the Convention, with a particular attention to positive implications

Recent Advances in Gene Editing and Synthesis Technologies and their Implications

Submitted by the United States of America

Summary

This paper considers several emerging biotechnology capabilities that may have implications for the Biological Weapons Convention (BWC), in view of their "dual use" implications for bioweapons development. It includes sections on: Gene editing; Metabolic pathway engineering; Gene drives; and Gene synthesis. The paper briefly describes some of the legitimate applications and benefits of these technologies, identifies challenges to realizing these benefits, and describes the nature and impact of their potential BW applications, to permit a balanced assessment. It then describes a recently developed framework for evaluating risks of misuse and identifying mitigation options. Finally, the paper highlights the need for greater international collaboration and harmonization of approaches to address potential biosecurity threats that might result from applications of biotechnology.

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I. Introduction

1. Advances in biotechnology, including the ability to engineer genomes of living organisms, bring many benefits to medicine, agriculture, industry, and the environment. At the same time, however, certain advances could expand the range of ways that biology could be used for harm. Biotechnology progress may, in some instances, challenge abilities to develop mechanisms to prevent or mitigate the risks of intentional misuse. Certain recent advances and their actual or potential applications have received a significant amount of attention, both positive and negative– but this attention has not always contributed to a nuanced and balanced understanding of either the potential or the limitations of these advances, nor to a similar understanding of their potential for misuse.

2. The August 2018 BWC experts meeting on advances in science and technology offers a timely opportunity to discuss emerging biotechnologies and consider potential risks, as well as explore foreseeable societal benefits. In some instances, advances in biological sciences may even facilitate the development of countermeasures that mitigate such risks or protect against biological threats. The goal for this analysis is to identify advances of particular concern to the BWC community, and to suggest approaches for future efforts to ensure that beneficial applications of biotechnology can continue to be used safely in medicine, agriculture, industry, and the environment by identifying and addressing risks of potential misuse.

II. Discussion of dual use biotechnology advances and related security concerns

Gene editing

3. Humans have manipulated the genes of natural organisms for thousands of years through intentional and unintentional artificial selection, including selecting for characteristics designed to increase food production of agriculturally important animals, crops, bacteria, yeast, and fungi. Improved understanding of the underlying mechanisms of heredity and the advent of chemical and radiation-based mutagenesis approaches in the early 20th century, followed by the development of recombinant DNA technology in the 1970's, enabled more targeted genetic manipulation of a wide variety of organisms.

4. Newer gene editing tools (including the CRSPR/Cas technology, and others that will continue to be developed) could enable a level of targeted and efficient genomic manipulation not possible previously. At this time, CRISPR-based tools offer greater precision; allow for multiple simultaneous gene edits as well as changing a single letter of genetic code; and are relatively inexpensive, available commercially, and straightforward to use by technical experts[1]. These gene-editing tools could benefit agriculture and the environment; for example, by increasing the ability of microbes to fix nitrogen, which would reduce environmental impacts of chemical fertilizer application[2]; by strengthening disease resistance in both crops and livestock, thereby reducing the application of pesticides; and by increasing desirable traits for sustainable food production and environmental resiliency. The U.S. Department of Agriculture recently approved an oilseed crop and a soybean variety for growth in the United States; both had been edited using CRISPR to produce more omega-3 oil and to be tolerant to drought, respectively.

5. In the health sector, clinical trials are already planned to use CRISPR to correct beta thalassemia and sickle cell disease, both inherited blood disorders caused by a mutation in a

[1] Thurtle-Schmidt, D. M., and Lo, T. W. Molecular biology at the cutting edge: A review on CRISPR/CAS9 gene editing for undergraduates. Biochem Mol. Biol. Educ., 46: 195-205 (2018).

[2] www.press.bayer.com/baynews/baynews.nsf/id/Bayer-Ginkgo-Bioworks-forces-sustainable-agriculture-forming-company-USD-million-Series-A

gene that makes hemoglobin. Chimeric antigen receptor (CAR) T cell therapy, a potentially remarkable new treatment that uses a person's own immune system to attack cancer cells, is also in development using CRISPR instead of viral vectors[3]. CRISPR-based and other newer gene editing tools can be used to selectively and reversibly "turn on" or "turn off" genes without changing the genetic code, significantly expanding the range of possible uses of this technology. Moreover, CRISPR technology could allow researchers to more readily modify not only adult ("somatic") cells, but to alter the DNA of heritable ("germline") cells – that is, to make genetic changes that will be passed on to offspring. This raises the possibility of potentially eradicating certain hereditary illnesses in animals or even humans, but also raises a number of important ethical and other considerations that are actively being discussed by the international scientific community, ethicists, and others.

6. Despite the many potential benefits of advanced biotechnologies, CRISPR/Cas and other gene editing tools could be intentionally misused for harmful applications, and thus could be an enabler for bioweapons development. For example, gene editing could be used to manipulate biological agents already of concern to the BWC. Historically, some bioweapons programs focused on altering naturally occurring pathogens to make them deadlier, make them spread more rapidly or easily, or to evade diagnosis and treatment – employing older gene editing tools and well-established recombinant DNA protocols. Newer gene editing approaches could be used for these same goals, arguably with fewer technical challenges than older approaches. Nevertheless, ensuring agent stability, achieving large scale agent production, and finding efficacious means of delivery remain the most challenging aspects of bioweapons development, regardless of the technology used to produce the agent, including by genetic manipulation. By itself, therefore, progress in gene editing may have a limited impact on the overall BW risk. The parallel development and convergence of other technologies (such as nanotechnology and automation) with genetic engineering approaches could lower barriers to weaponization by easing production and delivery challenges, and consequently increase the risk of misuse of gene editing.

Metabolic pathway engineering (a specific case of gene editing)

7. It is now possible to perform "metabolic pathway engineering"—that is, to genetically edit or introduce a novel mechanism into a microbe's internal metabolic production machinery. The creation of such cellular factories is a primary driver of synthetic biological manufacturing, a growing industry devoted to engineering organisms to create a variety of desired molecules in a more sustainable fashion, from biofuels to pharmaceuticals. Metabolic pathway engineering—even when using CRISPR/Cas technology—is technically challenging, and requires sophisticated tools for rational design and complex computation; however, advancement of genetic engineering tools and increasing accessibility also pose dual use concerns by lowering technical barriers and potentially enabling the creation of platforms to produce drugs, toxins, or other chemicals that could be misused or designed to escape traditional regulations for such commodities and their traditional production routes. Researchers have demonstrated the biological fermentation of opioids[4] and production of a marine conotoxin in the bacteria *Escherichia coli*[5], for example.

8. A small number of organisms have well-studied cellular pathways (for example, yeast and *E. coli*), but the list is likely to grow. Thus, the numbers of potential biological threat agents could expand and further complicate preparedness efforts and the ability to

[3] Eyquem, J., et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature, 543: 113-117 (2017).

[4] Galanie, S., et al. Complete biosynthesis of opioids in yeast. Science, 349: 1095–1100 (2015).

[5] Zhu, X., et al. Recombinant Expression and Characterization of α-Conotoxin LvIA in Escherichia coli. Marine Drugs, 14: 11 (2016).

mitigate public health concerns, or make the production of certain toxins cheaper and easier.

Gene drives (a specific application of biotechnology)

9. A gene drive is a way to "drive," or push, the prevalence of a gene in a sexually reproducing population so that a biased proportion of offspring will inherit a gene(s) of interest (in theory, close to 100% of offspring). There are many examples of gene drives that occur in nature (e.g., meiotic drives), but an *engineered* gene drive could make the addition, disruption, alteration, or suppression of specific genes within a target population of organisms a reality. As they require sexual reproduction and rapid life cycles to propagate, engineered gene drives would theoretically work best in insects and other organisms with short generation times and large numbers of offspring. Some proposed beneficial uses of engineered gene drives include eliminating disease transmission by mosquitoes, engineering crop pests to remove their resistance to pesticides, or making certain populations of invasive species like mice and other rodents infertile.

10. Resistance mechanisms to gene drives have been found[6], so the effectiveness of engineered gene drives for beneficial or malign purposes could be more complicated and less complete in a target population than previously thought. Also, gene drives are only relevant in species with sexual reproduction, so they could not be engineered to affect viruses and bacteria. While gene drives could, in theory, be used to alter human populations, the long generation time would make this an extremely long-term and unlikely proposition. In spite of these limitations, engineered gene drives hold dual use implications, as well as biosafety and biosecurity concerns. For example, engineered gene drive technology might be used to develop insects to intentionally spread diseases such as Zika or Dengue. The technology could also be used to alter populations of weeds or pests that harm crops or livestock by actors intent on harming food supplies.

11. It is key to note, however, that field trials of modified organisms containing an engineered gene drive have not yet occurred. Models have predicted a range of outcomes[7], from the evolution of resistance (ineffective drive) to effective drives that could have either intended or unintended consequences. Therefore, appropriate biosafety or biosecurity precautions should be followed to minimize the risks of such unintended consequences to the environment, as well as plant, animal, and human health. Notably, many of the potential environmental impacts of engineered gene drives are already being addressed by international fora other than the BWC.

Gene synthesis

12. The ability to synthesize DNA has become commonplace in both industrial and academic laboratories all over the world. The chemical synthesis of DNA oligonucleotides and their subsequent assembly into genes, pathways, and even entire genomes by synthetic biology methods has enabled biomedical research. DNA synthesis also enables the "design, build, test, and learn" cycle that underpins innovations in energy, agricultural, and health sectors. For example, the ability to produce vanilla from genetically modified yeast – rather than harvest the pure extract from vanilla beans – provides a cost-effective

[6] Champer, J., et al. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLoS Genet., 13: e1006796 (2017).

[7] Tanaka, H., Stone, H. A., and Nelson, D. R., Spatial gene drives and pushed genetic waves. Proc. Natl. Acad. Sci. USA, 114: 8452-8457 (2017); Eckhoff, P. A., et al. Impact of mosquito gene drive on malaria elimination in a computational model with explicit spatial and temporal dynamics. Proc. Natl. Acad. Sci. USA, 114: E255-E264 (2017); Turelli, M., and Barton, N. H., Deploying dengue-suppressing Wolbachia: Robust models predict slow but effective spatial spread in Aedes aegypti, Theor. Popul. Biol., 115: 45-60 (2017).

alternative with potentially lower environmental impact from unsustainable farming practices. Synthetic insulin, used by millions of diabetics, is made in both bacteria and yeast from DNA building blocks.

13. With decreasing costs to synthesize DNA, along with increasing demand for high fidelity DNA sequences, the gene synthesis industry has grown from a small handful of companies just a decade ago, to a marketplace of dozens of companies around the world, as well as into the hands of independent laboratories. The lengths of sequences that can be synthesized has continued to grow; the cost and time have continued to drop; and companies increasingly offer design services or tools to facilitate design (e.g., by optimizing expression of a given gene for the system in which it is to be inserted). Gene synthesis could therefore facilitate the creation of harmful biological agents "from scratch," produced outside of industry and other controlled commercial pipelines.

14. Although synthesizing genes is relatively straightforward, it is important not to overstate the ease of combining genetic elements to re-create harmful organisms *de novo*, as it is quite complicated; the level of difficulty is proportional to the size/length of the genomic DNA needed, and the overall complexity of the organism. There are also two steps required: the organism's genome must be synthesized in pieces and assembled, and then converted into a functioning biological organism, known as "booting." Re-creation and booting of a bacterial cell, although it has been accomplished[8], remains extremely technically challenging and, in many instances, expensive – and the recreation of eukaryotic organisms, such as yeast and fungi are, at present, not possible. However, synthesizing the genome of almost any mammalian virus is now possible, and sequences of most known human viruses are available on public internet databases such as GenBank, an annotated collection of all publicly available DNA sequences.

15. Experiments of this kind have already been performed, including the synthesis of polio virus and, more recently, the re-creation of the 1918 influenza virus[9] and the horsepox virus[10]. The publication of the smallpox virus genetic sequence in 1994[11]; creation of a bacterial cell with a synthesized genome in 2010[12]; and the synthesis and booting up of multiple viruses, including polio virus in 2002[13], a bacteriophage in 2003[14], and the 1918 influenza virus in 2005[9], were all legitimate research efforts. Although the creation and booting of some viruses (e.g., polio) has been achieved using cell-free extracts, most viruses must be booted inside cells, which requires additional time and technical expertise. Furthermore, some viruses, including horsepox virus, require the use of additional helper viruses and other specialized molecular tools, requiring considerable technical expertise, experience, and knowledge[15]. Synthesizing certain bacteria and toxins is also possible, although to a lesser extent than viruses, for several reasons both technical and scientific.

16. The capability to chemically synthesize or genetically engineer viruses poses biosecurity risks and should serve as a strategic warning to BWC States Parties that

[8] Hutchison, C. A., 3rd, et al. Design and synthesis of a minimal bacterial genome. Science, 351: aad6253 (2016).

[9] Tumpey, T. M., et al. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science, 310: 77-80 (2005).

[10] Noyce, R. S., Lederman, S., and Evans, D. H. Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. PLoS ONE, 13: e0188453 (2018).

[11] Massung, R. F., et al. Analysis of the complete genome of smallpox variola major virus strain Bangladesh-1975. Virology, 201: 215-240 (1994).

[12] Gibson, D. G., et al. Creation of a bacterial cell controlled by a chemically synthesized genome. Science, 329: 52-56 (2010).

[13] Cello, J., Paul, A. V., and Wimmer, E. Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. Science, 297: 1016-1018 (2002).

[14] Smith, H. O., et al. Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides. Proc. Natl. Acad. Sci. USA, 100: 15440-15445 (2003).

[15] DiEuliis, D., Berger, K., and Gronvall, G. K. Health Security, vol. 15 (Dec 2017).

biosecurity controls and preparedness – that rely primarily on controlling access to dangerous, existing pathogens – may be insufficient[16]. While many gene synthesis providers screen customers and orders for biosecurity concerns, following the recommendations of the industry-led International Gene Synthesis Consortium (IGSC) and/or the U.S. Department of Health and Human Services' *Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA*[17], such practices are not universal. Given that gene synthesis is performed by an array of international companies, and benefits legitimate research in many BWC State Parties, achieving greater safety and security around it will require discussions at international fora[18]. For example, dual use experiments which involve the creation of viruses for public health benefits (e.g., for the CAR T cell therapies mentioned above, or for vaccines) should be carefully reviewed for their risks, benefits, and safety – yet there is currently no broadly accepted set of norms for assessing risks and benefits of these experiments.

III. Science-based risk/benefit assessment as a tool for evaluating dual use capabilities

17. New biotechnology tools are benefiting human, agricultural, and environmental health, but also expanding capabilities for potential bioweapons development. As many scenarios are *possible*, it has been a difficult challenge for policy makers and regulators to focus on what might be *probable* or even feasible in a bioweapons context. In response to this problem, the United States government commissioned the U.S. National Academies of Sciences, Engineering, and Medicine to develop a framework for systematically evaluating the security implications of synthetic biology[19]. The Academy's study framework is focused on emerging, modern capabilities in biological engineering and is widely applicable. Factors for assessing the capability for malicious use of a biotechnology can include:

- The nature and capability of the technology itself;
- Its potential use as a weapon; i.e., how feasibly it could be weaponized, and its scope of damage or impact; and
- The attributes of actors who could command such a capability, including expertise, resources, and organizational footprint.

18. These factors must be weighed against the factors available for mitigation, which include:

- Deterrence and prevention of misuse;
- The ability to recognize a bioweapons attack has occurred;
- The ability to attribute an attack to the perpetrator; and
- The ability to provide for consequence management and recovery.

19. For example, while a particular agent may be possible to create in the laboratory it may be very difficult to deliver. Or, an agent to be re-created and delivered may already have a medical countermeasure available, possibly acting as a deterrent.

[16] DiEuliis, D., Gronvall, G. K. A holistic assessment of the risks and benefits of the synthesis of horsepox virus. mSphere, 3: e00074-18 (2018).

[17] www.phe.gov/Preparedness/legal/guidance/syndna/Pages/default.aspx

[18] DiEuliis, D., Carter, S. R., Gronvall, G. K. Options for synthetic DNA order screening, revisited. mSphere, 2: e00319-17 (2017).

[19] National Academies of Sciences, Engineering, and Medicine. Biodefense in the Age of Synthetic Biology. Washington, DC: The National Academies Press (2018). https://doi.org/10.17226/24890.

20. Importantly, these factors are related to risk analysis – or, in the least, generating a level of concern for particular scenarios enabled by emerging biotechnology. Overall, these concerns should be weighed against the potential benefits proposed by the research. Balancing risks and benefits can be a fraught process, as exemplified by recent controversy surrounding "gain of function" avian influenza research. As a subset of research in areas where there is potential for misuse, gain of function research is being defined in this context as experimentation that could increase the transmissibility and/or virulence of pathogens. Thus, the benefits to pandemic preparedness (prediction, surveillance, detection, and treatment) must be weighed against the risk of creating potential pandemic agents in the process that could be used for harm, or unintentionally released. A summary of such discussions can inform the risk/benefit analysis process[20].

IV. Converging international approaches for ensuring safe and secure use of emerging biotechnologies

21. Perhaps not surprisingly, scientific and technological advances in recent years in the biosciences have been accompanied by concerns about the possible misuse of research results for malign applications. Preventing deliberate misuse of these advances in the biological sciences is, of course, a key aim for the BWC States Parties. Vigorous discussions are taking place in the scientific community and government in the United States and in other international and domestic fora to assess the risks of emerging biotechnologies and to develop mechanisms for preventing or mitigating misuse while, importantly, not hindering legitimate research and beneficial applications.

22. For example, the Interacademy Partnership (IAP), representing more than 130 national and regional member academies of science, sponsored a workshop in October 2017 entitled "Assessing the Security Implications of Genome Editing Technology"[21]. While international discussions are still at an early stage, the IAP identified a number of key messages from the workshop:

- Recent evidence confirms that genome editing will be an important tool to drive innovation in pursuit of societal priorities.
- As with other tools, it could be misused, inadvertently or deliberately. While the advantages of genome editing lead to its widespread use, this does not in itself directly promote intent to misuse. There must be balanced discussion about benefit and risk, valuing benefits in ways that are relevant to the public. Benefits may include increasing security for human health and agriculture.
- [The] workshop discussions represent significant progress in bringing together members of the science, security and policy communities to clarify if, where and how intentional misuse can be expected and what we might do to prepare for and mitigate such eventualities. We must listen to concerns about misuse while also making clear what is, or is not, scientifically feasible. We must continue building a culture of research responsibility and integrity, knowing that uncertainty may undermine public confidence in science and that other stakeholders may have different expectations of evidence.
- The voices of our countries worldwide are essential in our collective efforts to assess value and harmonize procedures for risk assessment and management. It is highly desirable to build on the evidence shared and the links formed in [the] workshop to develop a sustainable network encompassing the scientific and

[20] Institute of Medicine and National Research Council. Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop. Washington, DC: The National Academies Press (2015). https://doi.org/10.17226/21666.

[21] www.interacademies.org/43251/Assessing-the-Security-Implications-of-Genome-Editing-Technology-Report-of-an-international-workshop

security communities as a basis for extending the engagement more widely. IAP is ready to continue playing its part in doing this.

23. The United States considers that the issue of potential misuse of biotechnological advances is not unique to advances in gene editing. For example, gain-of-function influenza research may make use of advanced gene editing techniques, or may use simpler expedients, such as serial passage through numerous animal specimens, as a means of acquiring a desired trait. The United States government has developed guidelines governing research that may be considered gain-of-function (now "potential pandemic pathogen care and oversight"[22]). In addition, the United States government has policies on the oversight of "dual use research of concern" (DURC)[23], which seek to preserve the benefits of life sciences research while minimizing the risk of misuse of such knowledge or technologies. A number of other countries have developed similar guidelines. The United States welcomes international discussions in the BWC and elsewhere, and looks forward to continuing to play an active part in further international efforts at sharing and harmonizing best practices and norms around biosecurity.

24. In addition to deliberate misuse of advances in the biosciences, safety mishaps, inadvertent misuse, or negligence could also lead to unintended consequences. In some scenarios, safety failures might even lead to a Public Health Emergency of International Concern (PHEIC), as defined by the World Health Organization. When such a disease event is first detected and reported, it may also be difficult to determine whether the potential PHEIC is the result of deliberate misuse, a natural emergence, or an accident.

V. Conclusion

25. To ensure that countries continue to apply advances in biotechnologies for peaceful purposes and, in turn, reap the undoubted benefits that these technologies otherwise afford, there is a need for countries to develop and implement biosafety and biosecurity policies and approaches, and to share these internationally among BWC States Parties. The development of measures for ensuring safety and security of biological laboratories, as well as for preventing or mitigating intentional or unintentional misuse of biotechnology, is traditionally a national matter. Nations have their own laws and regulations regarding handling and protection of pathogens, and preventing misuse of research findings. Given increasing international research collaborations and the potential global consequences of misuse of advances in the biosciences, it is desirable to discuss and find ways to harmonize national practices. Examples of successful harmonization and international adoption of biosecurity policies include - together – the approaches to managing gene synthesis risks taken by the U.S. Department of Health and Human Services' *Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA*, the IGSC, and the Australia Group. Continuing and expanding such efforts will require close cooperation between the BWC States Parties and the international scientific community.

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[22] www.phe.gov/s3/dualuse/Pages/p3co.aspx

[23] https://osp.od.nih.gov/biotechnology/dual-use-research-of-concern/