Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

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Advances in science and technology: Production and delivery

Submitted by the United States of America

I. Introduction

1. This year's topic of discussion under the science and technology (S&T) agenda item is, "Advances in production, dispersal and delivery technologies of biological agents and toxins." In Sections II and III, we review specific examples of selected S&T advances pertaining to production and delivery published during the past year. Key technical terms are underlined and defined in the text or in footnotes. Specifically, we describe advances that make possible the production of a novel product in bacteria, a yeast strain with an entirely synthetic chromosome, and delivery of vaccines using microneedle technology. Upon review, we conclude that these S&T advances exemplify peaceful uses that aim to benefit humankind and likely do not pose transformative risks for uses contrary to the Convention.

2. However, as discussed in Section IV, any assessment of risk posed by new scientific knowledge and technology requires a comparison to the established knowledge and technology. If risk is the product of likelihood and consequence, then we must also learn the incentives (and disincentives) for using new technologies and how the consequence of their use differs from the consequence of using established technologies. Such deliberations will require scientific expertise; we suggest that a working group of scientific experts could perform the relevant analyses and keep delegations up to date on the latest scientific advances with potential implications for the Convention.

3. While this paper is modeled upon the one we submitted to last year's MXP, this year we have provided more background information in effort to increase understanding and awareness of the essential scientific concepts underlying the latest laboratory research. The delegation of the United States of America seeks feedback from States Parties regarding the clarity, relevance and usefulness of this paper, as well as suggestions for its improvement.

II. Production

4. Production entails growing microorganisms in large quantities and purifying them to isolate the microorganisms themselves or a product made by them. Recent advances in synthetic biology[1] make it possible to engineer[2] microorganisms so they grow more efficiently or produce novel products. We discuss below two articles published in 2014 that describe the engineering of two microorganisms for production purposes: bacteria and yeast.

5. **Engineering bacteria**. Bacteria are single-celled microorganisms with rudimentary internal structures, including a single chromosome.[3] The bacterium *Escherichia coli* (abbreviated as *E. coli*) was one of the first species used in early genetic engineering studies in the 1970s. A significant early achievement was the large-scale production of human insulin derived from engineered *E. coli* bacteria in the 1980s.[4] Before this, diabetics relied on insulin extracted from livestock, but the extraction process was inefficient – 2000 kilograms of pancreas (the organ that produces insulin) would yield only about 250 milliliters of insulin.[5] Over the past 30 years, new strains of *E. coli* have been engineered for specialized production of complex or rare materials.

6. **Production of chondroitin in metabolically engineered *E. coli***. For example, scientists recently engineered *E. coli*'s natural processes to make large quantities of a complex product – a process known as metabolic engineering. In this study,[6] the desired product was chondroitin sulfate (CS), a naturally occurring component of cartilage important for the structural integrity of joints in the body. Taken orally, CS is thought to prevent degradation of cartilage and may even promote growth of new cartilage. For these reasons, CS is used as an anti-inflammatory drug for arthritis patients. As was the case 30 years ago for insulin, animal tissues are the current sources of CS – but unlike insulin, chondroitin is a large, complex molecule requiring multiple metabolic steps to produce. To create an alternative, non-animal source of CS, the scientists added three genes to a non-pathogenic *E. coli* strain – these genes encode enzymes[7] that catalyze the step-by-step formation of CS inside the bacterial cells. They then optimized the growth conditions of *E. coli* containing these enzymes in order to maximize the yield of CS. Work remains to be done to increase the efficiency of CS production in *E. coli*. For example, in this study most of the CS produced remained stuck to the bacterial surface instead of being released into the liquid culture medium, from which it is easier to purify. Nonetheless, this study

[1] There is a variety of definitions for synthetic biology, several of which are listed at http://www.synbioproject.org/topics/synbio101/definition/. Let us use here the simplest of these definitions: "Synthetic biology is a) the design and construction of new biological parts, devices and systems and b) the re-design of existing natural biological systems for useful purposes."

[2] To engineer a microorganism is to alter its DNA to produce some desired effect.

[3] A chromosome is a single, continuous piece of DNA found naturally in living organisms.

[4] Johnson IS. (11 February 1983) Human insulin from recombinant DNA technology. *Science* 219(4585):632-7. Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/6337396.

[5] Gebel E. (July 2013) Making Insulin: A behind-the-scenes look at producing a lifesaving medication. Full text article available at http://www.diabetesforecast.org/2013/jul/making-insulin.html.

[6] He W, Fu L, Li G, Andrew Jones J, Linhardt RJ, Koffas M. (January 2015) Production of chondroitin in metabolically engineered E. coli. *Metabolic Engineering* 27:92-100. doi: 10.1016/j.ymben.2014.11.003. Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/25461828.

[7] Enzymes are proteins that accelerate chemical reactions. The three-dimensional structure, or shape, of an enzyme determines its function. For example, the enzyme maltase fits the shape of the molecule maltose and breaks it into two molecules of glucose. Enzymes are required for many of the chemical reactions that take place inside a cell.

demonstrates that complex molecules like CS can be synthesized in *E. coli* and, with further optimization, offers a potential alternative to animal sources for this drug. Metabolic engineering has also been applied recently[8] to synthesis of farnesene, an oil found naturally in plants that has many industrial applications, including pharmaceuticals, fragrances, adhesives, and biofuels.

7. **Engineering yeast**. Yeast are single-celled microorganisms that have internal structures similar to animal cells. The yeast species *Saccharomyces cerevisiae* has been used by humans for thousands of years to make products like bread and beer. As with bacteria, yeast can be grown in large numbers to harvest the cells themselves or a product that the cells produce. For example, large numbers of yeast cells are harvested and dried to produce baker's yeast, whereas in beer production, yeast cells are grown in a nutrient-rich medium of grains and water to produce ethanol. In recent years, scientists have engineered *Saccharomyces cerevisiae* to expand the types of products this yeast species can produce, like the antimalarial drug artemisinin and the chemical precursors of morphine.[9]

8. **Total Synthesis of a Functional Synthetic Eukaryotic Chromosome.** In 2014, scientists published a paper describing the construction of a synthetic chromosome in yeast cells.[10] The authors are part of an international consortium that aims to replace each of the yeast's 16 chromosomes with synthetic ones.[11] Undergraduate students assembled small pieces of DNA (ordered from a commercial DNA synthesis firm) into progressively larger fragments. The ends of these large fragments were tagged with a signal that yeast naturally use to recombine their own DNA in a process called homologous recombination.[12] When the tagged large fragments were put into yeast cells, the yeast incorporated these synthetic fragments into the natural chromosome. This process was repeated until the entire length of yeast chromosome 3 was replaced with the synthetic fragments. This synthetic chromosome was demonstrated to be fully functional and inheritable, with little to no adverse impact on the growth and survival of yeast cells in culture. This is a proof-of-principle study showing the feasibility of swapping a natural microbial chromosome for a synthetic one. In theory, this means that microorganisms like yeast will tolerate changes to large expanses of DNA, suggesting they can be engineered to produce a greater number of complex products in the future. Similar advances have been demonstrated in bacterial cells, which were able to function and replicate with a wholly synthetic chromosome.[13]

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[8] Zhu F, Zhong X, Hu M, Lu L, Deng Z, Liu T. (July 2014) In vitro reconstitution of mevalonate pathway and targeted engineering of farnesene overproduction in Escherichia coli. *Biotechnology and Bioengineering* 111(7):1396-405. doi: 10.1002/bit.25198. http://onlinelibrary.wiley.com/doi/10.1002/bit.25198/abstract

[9] Ehrenberg R. (18 May 2015) Engineered yeast paves way for home-brew heroin, *Nature* 521(7552):267-268. doi:10.1038/251267a. Full text article available at no cost at http://www.nature.com/news/engineered-yeast-paves-way-for-home-brew-heroin-1.17566.

[10] Annaluru N, Muller H, Mitchel LA et al. (4 April 2014) Total Synthesis of a Functional Designer Eukaryotic Chromosome, *Science* 344(6179):55-58. DOI: 10.1126/science.1249252. Full text article available at no cost at http://www.sciencemag.org/content/344/6179/55.full.

[11] Pennisi E. (23 March 2014) Building the Ultimate Yeast Genome, *Science* 343(6178):1426-1429. Full text article available at no cost at http://www.sciencemag.org/content/343/6178/1426.full.

[12] http://www.nature.com/subjects/homologous-recombination

[13] Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C, Vashee S, Krishnakumar R, Assad-Garcia N, Andrews-Pfannkoch C, Denisova EA, Young L, Qi ZQ, Segall-Shapiro TH, Calvey CH, Parmar PP, Hutchison CA 3rd, Smith HO, Venter JC. (2 July 2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science*, 2:329(5987):52-6. doi: 10.1126/science.1190719. Full text article available at no cost at http://www.ncbi.nlm.nih.gov/books/NBK84435/?report=printable.

III. Delivery

9. Delivery entails providing drugs or vaccines to patients orally, by injection, by inhalation, or by application to the skin or mucosal surface.[14] A key objective for medical and pharmaceutical professionals is to maximize drug efficacy and delivery efficiency while minimizing their side effects. Optimized delivery systems can more effectively deliver drugs and vaccines to the targeted site in specific tissues where they work, at a controlled rate, using methods least harmful to the human body. Improving traditional delivery methods enhances the use of existing drugs or vaccines and may reduce associated costs.

10. In recent decades, drug delivery has become one of the most active areas of biomedical research. Exciting advances in genomics[15] and systems biology[16] continue to reveal new therapeutic targets to treat diseases, while innovations in chemistry and materials science have yielded biodegradable and nontoxic carriers for targeted delivery. In addition, nanotechnology developments enable precise control of the shape and size of nanoparticles, which is expected to stimulate the applications of these particles as drug delivery devices. We discuss below a recent example of the use of microneedles in a vaccination study. Microneedles are needles less than one millimeter in length, are typically arranged in rows, and can be made of metal or synthetic polymers. Drugs or vaccines can be coated on the surface of solid microneedles, injected via hollow microneedles, or impregnated in polymer microneedles that dissolve.[17]

11. **Vaccination with human papillomavirus targeted to skin using microneedles.** In March 2015, scientists published a study in the open access journal *PLoS One* demonstrating effective microneedle delivery of vaccine against the human papilloma virus (HPV).[18] HPV (specifically the strain HPV16) is the primary cause of cervical cancer. Human papilloma virus-like particles[19] (HPV VLP) serve as the basis of the current licensed vaccines for HPV. These VLPs have an outer "shell" composed of two proteins naturally found in HPV. Because these proteins are very efficient at binding to mucosal surfaces, they are also efficient at binding to similar types of cells – called epithelial cells – found in skin. This makes HPV VLPs well suited to vaccination targeted to the skin using

[14] In the body, a mucosal surface is one where tissues are exposed to the external environment. These surfaces are lined with specialized cells that regulate whether substances from the external environment pass into the body or are excluded from it. Respiratory, digestive and genito-urinary tracts are lined with such specialized cells.

[15] A genome is the complete set of genetic material within an organism or microorganism, including its genes. Genomics is a field of study concerned with the structure and function of genomes, including genome sequences.

[16] There is a variety of definitions for "systems biology" (e.g., see this *Nature* blog entry http://blogs.nature.com/sevenstones/2007/07/what_is_systems_biology_3.html). Let us define it here as a scientific approach to understand phenomena by looking separately at the affected biological systems and developing computer models representing relationships among the affected systems.

[17] Prausnitz MR, Mikszta JA, Cormier M, Andrjanov (4 August 2009) Microneedle-based Vaccines. Current Topics in Microbiology and Immunology, 333:369-393. Full text article available at no cost at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2904604/.

[18] Kines RC, Zarnitsyn V, Johnson TR, Pang YY, Corbett KS, Nicewonger JD, Gangopadhyay A, Chen M, Liu J, Prausnitz MR, Schiller JT, Graham BS. (18 March 2015) Vaccination with human papillomavirus pseudovirus-encapsidated plasmids targeted to skin using microneedles. *PLoS One*, 18;10(3):e0120797. doi: 10.1371/journal.pone.0120797. Full text article available at no cost at http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0120797.

[19] Virus-like particles (VLPs) are structurally similar to viruses but have been engineered to contain only desired genetic material.

microneedles. In this study, the scientists demonstrated that HPV VLPs delivered by microneedles induced a protective immune response. The study also demonstrated that HPV VLPs could be used to deliver vaccine against another virus (called respiratory syncytial virus, or RSV) by loading RSV DNA into the HPV VLP "shells." Finally, the scientists point out in their article, "Use of microneedles could eliminate the need for typical needle injections and provide a simpler, less painful, safer and potentially more cost-effective approach to vaccination by eliminating the need for cold-chain transportation and storage."

IV. Implications for the BWC

12. Using microorganisms to produce novel materials is desirable for several reasons: 1) they quickly multiply to large numbers, 2) they can be engineered to produce versions of products that are safer and more effective for humans, 3) the engineering of microorganisms is more socially and ethically acceptable than using or engineering animals for production, and 4) their potential to increase production of rare natural products could lower costs. Advances in production technologies such as those described above exemplify creative, peaceful uses that aim to benefit humankind.

13. Advances in technology to improve delivery of drugs and vaccines could offer several advantages. For example, use of microneedles would reduce material requirements and waste (no separate syringe and vial necessary), provide a surface to stabilize the drug or vaccine, and allow for efficiencies due to a lack of need for cold storage (access to more remote locations). Technologies that improve delivery may ultimately result in higher vaccination rates[20] and enable self-administration of drugs that would otherwise require visits to a healthcare professional.

14. Theoretically, these technologies could also be used to produce or administer products harmful to humans. It has long been possible to purify toxins from wild-type[21] bacteria and microorganisms themselves were grown and stored in large quantities for past state-sponsored offensive biological weapons programs. When faced with new scientific knowledge and technology, States Parties should critically ask, How might these new developments increase risk of harm to humans compared to older, established knowledge or technology? What are the incentives and disincentives for using a new technology, what are the consequences that accompany its use, and how do they compare with consequences of the older, established technology? In other words, we must seek to identify **transformative** knowledge and technology that is likely to be different enough from the status quo as to significantly change our calculation of risk.

15. Article XII directs States Parties "to review the operation of the Convention," and that "[s]uch review shall take into account any new scientific and technological developments relevant to the Convention." For the 2014 and 2015 Meetings of Experts, we have undertaken a review of the scientific literature and reported key examples of research relevant to the year's S&T topics in working papers like this one. Our experience to date has been instructive in two respects. First, it has inspired our confidence that an expert S&T working group can thoroughly review the relevant scientific literature and, through reports and presentations, keep delegations up-to-date on the latest scientific advances with potential implications for the Convention. Second, it suggests that routine review of the scientific literature in search of relevant advances is just the beginning of a process that

[20] http://www.cdc.gov/media/releases/2015/p0427-microneedle-patch.html

[21] Wild-type is a term used to describe an organism whose genome can be found in nature and has not been engineered in any way.

would enable States Parties to reach common understandings and take effective action. Beyond any review of new knowledge and technology lie additional questions: Have expanded knowledge and technology changed the risk of harm by making biological weapons more virulent, cheaper or easier to produce? Will these advances somehow embolden bad actors? And do answers to any of these questions suggest "effective actions" that States Parties should undertake?

16. Deliberations like these require scientific expertise, high-quality data, time, and effort. We suggest that an expert S&T working group, equipped with the highest quality data available, could perform useful analyses to support States Parties as they consider these essential questions. If the products of an expert S&T working group promote common understanding of the technical details, then States Parties could focus on effective actions if risk is assessed to be increased by particular new knowledge or technology.