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Advances in science and technology: production, dispersal and delivery technologies

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Summary

This working paper considers some examples of recent developments in science and technology relevant to the 2015 topical scientific subject 'advances in production, dispersal and delivery technologies of biological agents and toxins'. Contributions relevant to these topics provided in previous science and technology reviews should also be taken into account. Examples of progress in production technologies include fermentation systems, transgenic animals and plants, peptide synthesis, 3-D printing and portable containment systems. Advances in dispersal technologies include the use of UAVs for aerial spraying, aerosol characterisation techniques, and biological control measures. Various approaches to the design of vaccine and therapeutic delivery systems are described, including by utilisation of nanomaterials. The benefits and risks of such advances for the BTWC are discussed under the heading "Implications and Conclusions": possible applications for hostile purposes are clearly prohibited by the Convention, while there are potentially highly beneficial outcomes for combating disease and improving agricultural production. The importance of bringing in relevant experts to assist in discussion of key advances is emphasised, and it is recommended that efforts to promote greater collaboration with the CWC be continued in light of the growing convergence between the fields of chemistry and biology

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I. Introduction

1. The topical scientific subject of 'advances in production, dispersal and delivery technologies of biological agents and toxins' to be addressed in 2015 has been considered in previous contributions to the review of scientific and technological developments relevant to the Convention by the United Kingdom and by other States Parties. The UK science and technology review paper submitted to the Seventh Review Conference included sections on advances in biological production technologies and on delivery and dispersal.[1] In 2013, we submitted a working paper that summarised recent developments in vaccine design, production and delivery and considered their implications for the BTWC.[2] Developments that contribute to capabilities to design and produce biological agents with altered characteristics have been discussed throughout the intersessional programme standing agenda item on review of developments in science and technology, for example when considering enabling technologies and advances in the understanding of pathogenicity, virulence, toxicology and immunology.[3] The relevant implications outlined in previous reviews should be taken into consideration during this year's discussions. This working paper provides a brief summary of some more recent relevant advances in these fields.

II. Production

2. Single-use or disposable bioreactor systems are in increasing demand for commercial production processes, offering smooth transfer from process development to manufacturing scale. Recently available systems are reported to have the capability to achieve cell growth rates and productivity comparable to traditionally stirred fermenters, but with all the advantages of single-use technology, such as quicker turnaround time, contamination risk reduction, operational flexibility, reduced validation requirements, no need for cleaning and sterilization and significant cost savings. The availability of a range of accessories and options means that they can be equipped for cell culture or microbial fermentation processes. Advanced automation and modular system design can allow several bioreactors to be controlled from a single control unit, ensuring process control and data management.

3. Developments in transgenic animals and plants as a means of producing therapeutics and vaccines have continued. Several examples were described in the 2013 UK working paper on vaccine development.[4] Recent studies report the engineering of transgenic goats to produce milk with enhanced antimicrobial properties to prevent diarrhoea, malnourishment and child mortality.[5] Prototype Good Manufacturing Practice-compliant processes and facilities for production of pharmaceutical proteins in transgenic plants have been reported. These take advantage of well-established tools for optimising protein expression to get products to the manufacturing stage in highly competitive time periods. Features of the system include rapid production cycles, high product yield, virtually unlimited scale-up potential, and flexibility for different manufacturing schemes, such as fast production of

[1] BWC/CONF.VII/INF.3/Add.1 pages 31-33

[2] BWC/MSP/2013/MX/WP.8

[3] For example, see BWC/MSP/2012/MX/INF.1; BWC/MSP/2012/MX/WP.14; BWC/MSP/2014/MX/WP.2; BWC/MSP/2014/MX/WP.4

[4] BWC/MSP/2013/MX/WP.8 pages 3-4

[5] Bertolini et al (2014) Transgenic animal models for the production of human immunocompounds in milk to prevent diarrhea, malnourishment and child mortality: perspectives for the Brazilian Semi-Arid region. BMC Proceedings 8(Suppl 4)O30

small batches of individualised pharmaceutical proteins and large scale production of therapeutic antibodies.[6] A recent example of therapeutic production in transgenic plants is ZMapp, an experimental drug used to treat several aid workers infected during the Ebola Virus Disease (EVD) outbreak in West Africa. It comprises three monoclonal antibodies produced in transgenic tobacco plants and is reported to be readily scalable to mass production under GMP-compliant conditions.[7]

4. The UK working paper on 'The convergence of chemistry and biology: implications of developments in neurosciences', submitted to the 2012 Meeting of Experts, highlighted the relevance of advances in peptide production to both the BTWC and the CWC.[8] It noted the commercial synthesis of small peptides, including toxins, and the need to assess the practical limitations associated with the technical ability to chemically synthesise toxins, bio-regulators and biologically active peptides. Recent advances in the field, including process improvements, more favourable sourcing of raw materials and solvents, and advanced protection and coupling chemistries have led to lower costs and to synthetic chemistry predominating peptide drug manufacture; however, purification and lyophilisation remain costly. Continuing improvements in peptide manufacturing have given rise to complex products, some branched, some containing artificial amino acids.[9] A 'synthetic fermentation process' has recently been developed to produce large libraries of bioactive, non-natural peptides from small building blocks in water, without the use of organisms, enzymes or reagents. The 'fermentation' products can be screened directly for biological activity without any purification.[10] In this proof-of-concept study, around 6000 unnatural peptides were produced from just 23 building blocks, and screened to identify and characterise biologically active molecules for the inhibition of hepatitis C virus protease, which is believed to play a key role in viral replication.

5. 3-D printing is an emerging technology of potential future relevance; a recent workshop on the convergence of chemistry and biology reported that it would be years before a substantially larger printing capacity would be available.[11] However, developments in this technology should continue to be monitored since there may be implications for the production of biological agents and toxins in the future. A 3D-printing technology used to assemble mammalian cells could also be applied to 'printing' or assembling bacterial biofilms and enable studies to increase understanding of the interactions within such bacterial aggregates and characteristics such as enhanced antibiotic resistance and the impact on infection.[12] A 3-D laser printer technology has been developed to produce DNA on a commercial basis, with the potential to make it significantly quicker and more affordable. 3-D printing could also potentially be used to make equipment or components utilised in biological production.

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[6] Klimyuk et al (2014) Production of recombinant antigens and antibodies in Nicotiana benthamiana using 'magnifection' technology: GMP-compliant facilities for small- and large-scale manufacturing. In Plant Viral Vectors - Current Topics in Microbiology and Immunology 375:127-154

[7] Zhang et al (2014) Fighting Ebola with ZMapp: spotlight on plant-made antibody. Science China Life Sciences 57:987-988

[8] BWC/MSP/2012/MX/WP.1

[9] DePalma A (2015) Peptides: New Processes, Lower Costs. Genetic Engineering and Biotechnology News July 2015: 24-26

[10] Huang Y-L & Bode J.W (2014) Synthetic fermentation of bio-active non-ribosomal peptides without organisms, enzymes or reagents. Nature Chemistry 6:877-884

[11] Spiez CONVERGENCE Report on the first workshop 6 – 9 October 2014. http://www.labor-spiez.ch/en/akt/pdf/Spiez_Convergence_2014_web.pdf

[12] Connell et al (2014) Real-time monitoring of quorum sensing in 3D-printed bacterial aggregates using scanning electron microscopy. Proceedings of the National Academy of Sciences 111:18255-18260

6. Biological containment facilities have become increasingly accessible to the wider scientific community in recent years, with increased calls for research on pathogens. There have also been increasing drivers for portable biological containment systems: portable Class III cabinets and gloveboxes, as well as mobile laboratories housed in vans, trucks and trailers, are readily available. Such facilities enable the production of biological agents away from designated and controlled high containment facilities. During infectious disease outbreaks, such as the EVD outbreak in West Africa, portable biocontainment systems can be quickly distributed to locations where there are no scientific centres with appropriate capabilities.

III. Dispersal

7. Research on aerobiology directed at optimising agricultural spraying techniques has included field trials on the use of unmanned aerial vehicles (UAVs). Results have shown that spray application and deposition rates were comparable to those obtained in manned aerial spraying and ease of deployment was also demonstrated, leading to the conclusion that UAVs can be successfully deployed in speciality crop spraying.[13] Techniques for analysis of aerosolised droplets have also been investigated to determine the optimal methods to provide accurate droplet size from spray nozzles for use in efficacy testing and drift assessments.[14]

8. Studies have been conducted recently on the survival and deposition of microorganisms in the atmosphere.[15] Information obtained is of importance for modelling the dispersal of microbial disease and for determining the limits of the dispersion of microorganisms through the atmosphere depending on environmental conditions. In addition, developments in modelling have facilitated measurement of human exposure to airborne spray and ground deposits.[16]

9. Successes in biological control measures using antagonistic agents against plant pathogens continue to lead to the manufacture and registration of biological control products, which minimise the need for use of chemical pesticides. Recent work has demonstrated the potential of a new oil-based formulation of a biological control agent that provides improved viability and persistence on the target crop. As well as the positive environmental benefits, this formulation was found to be well suited to the types of crop sprayers used in the field.[17]

Delivery

[13] Giles et al (2014) Deployment and performance of an unmanned aerial vehicle for spraying of speciality crops. Proceedings International Conference of Agricultural Engineering, Zurich, 06-10.07.2014
Xinyu et al (2014) Drift and deposition of ultra-low-altitude and low volume application in paddy field. International Journal of Agriculture and Biological Engineering 7(4):23-28

[14] Fritz et al (2014) Measuring droplet size of agricultural spray nozzles – measurement distance and airspeed effects. Atomization and Sprays 24(9):747-760

[15] DeLeon-Rodriguez et al (2014) Microbiome of the upper troposphere: species composition and prevalence. Effects of tropical storms, and atmospheric implications. Proceedings of the National Academy of Sciences 110(7):2575-2580
Hurtado et al (2014) Characterization of atmospheric bioaerosols at 9 sites in Tijuana, Mexico. Atmospheric Environment 96:430-436

[16] Van de Zande et al (2014) Spray drift and bystander risk from fruit crop spraying. Aspects of Applied Biology 122:177-185

[17] Mbarga et al (2014) A new oil-based formulation of *Trichoderma asperellum* for the biological control of cacao black pod disease caused by *Phytophthora megakarya*. Biological Control 77:15-22

10. Some recent developments in vaccine delivery were described in the 2013 UK working paper on advances in vaccine development, including nanotechnology-based approaches. Advances in this area have continued and include the development of a promising nanoparticle system for needle-free vaccine delivery to the lungs by aerosol or nasal delivery. In this system, the protein fragments that constitute the vaccine are encased in a nanocapsule made up of interconnected layers of lipids that protect it from disintegration long enough to stimulate an immune response when it reaches the lungs.[18]

11. The use of many other nanomaterials in drug delivery has been explored, and potential benefits such as reduced dosing, enhanced solubility and targeted delivery have been highlighted. However, safety aspects are of concern, and the balance between maximising therapeutic benefit while minimising toxic potential is a significant challenge in development of new targeted nanomedicines.[19] The use of graphene materials in drug delivery has been investigated; these applications exploit properties such as high specific surface area and hydrophobic interactions for loading small molecule drugs.[20] Nanomaterial based approaches also have the potential for delivery of genetic elements, for example in gene therapy. Recent studies have reported the potential use of gelatin nanoparticles for the targeted delivery of medicines to the brain. These can be administered nasally, providing a non invasive and direct route to the brain, by-passing the blood-brain barrier.[21]

12. DNA origami involves folding strands of DNA into complex 3-D shapes, and has emerged as a powerful method for the design and fabrication of self-assembled nanodevices. A recent publication reports the use of a biomolecular system to mimic large-scale engineering systems in developing molecular machines, making flexible parts from single-stranded DNA and more rigid parts from double-stranded DNA.[22] This allowed the machine's movements to be reversible and repeatable. Such structures have the potential to transport biomolecules to targets within the body and thus for development as drug delivery systems.

13. In a different approach to drug delivery, researchers have taken advantage of increased understanding of the mechanism by which anthrax toxin enters cells. The protective antigen component of the toxin, which binds to mammalian cell receptors and forms a pore to allow the toxic components to penetrate the cell, was isolated and bound to other proteins. These proteins, known as antibody mimics, could then be delivered effectively into target cancer cells, where they act by disrupting protein-protein interactions to destroy the cells.[23]

[18] Li et al (2013) Generation of effector memory T-cell-based mucosal and systemic immunity with pulmonary nanoparticle vaccination. Science Translational Medicine 5(204), pp.204ra130

[19] Palombo et al (2014) Pharmaceutical and toxicological properties of engineered nanomaterials for drug delivery. Annual Review of Pharmacology and Toxicology 54:581-598

[20] Goenka et al (2014) Graphene-based nanomaterials for drug delivery and tissue engineering. Journal of Controlled Release 173:75-88

[21] Joachim et al (2014) Gelatin nanoparticles enhance the neuroprotective effects of intranasally administered osteopontin in rat ischemic stroke model. Drug Delivery and Translational Research 4(5-6):395-399

[22] Marras et al (2015) Programmable motion of DNA origami mechanisms. Proceedings of the National Academy of Sciences 112:713-718

[23] Liao et al (2014) Delivery of antibody mimics into mammalian cells via anthrax toxin protective antigen. ChenBioChem 15:2458-2466

IV. Implications and conclusions

14. As with many fields in the life sciences and related subjects, some advances described here have the potential for uses contrary to the provisions of the Convention. Developments in production technologies which improve yield, speed, flexibility, cost-effectiveness, portability, availability and safety would also be applicable for the production of biological or toxin weapons agents. Improved dispersal and delivery methods also have the potential to be misused for hostile purposes, and information from modelling systems could be used to inform the development of methods for the dissemination and delivery of biological or toxin agents. However, such offensive applications are clearly prohibited by Article I of the Convention and States Parties have a commitment to take the necessary national measures to prohibit and prevent the misuse of science. Measures for the governance and oversight of such advances need to be designed to prevent prohibited activities without having adverse effects on legitimate developments. This is relevant to discussion under the standing agenda item on national implementation, as well under the science and technology topics on biological risk management, codes of conduct and education and awareness-raising.

15. Scientific and technological developments in production methods for biological agents and toxins may have implications for any future review of Confidence Building Measures. In particular, emergence of new production methods that are not based on traditional fermentation methods, or do not need to be performed in containment facilities would be of relevance to the types of facilities of most interest in the future.

16. However, it is clear that the most significant outcome of advances in these areas is the potential benefit in combating disease and in agriculture and food production; such examples embody the prophylactic, protective and other peaceful purposes for biological agents and toxins stated in Article I. The development of improved methods to design and produce vaccines and therapeutics rapidly and cost-effectively, and to increase efficient delivery within the host, will have significant impact on global efforts in preventing and responding to infectious disease outbreaks. Novel delivery methods may also be of relevance for therapy of many other diseases, for example, cancers, neurological diseases, metabolic and genetic disorders. Advances of relevance to agriculture, such as improved biological control methods to combat plant pests and diseases, and approaches to improve production of food components, also have the potential for global impact. These beneficial aspects are also highly relevant to the BTWC. Capabilities to prevent, prepare for and respond to infectious disease outbreaks, whether natural, accidental or deliberate are important for the implementation of Article VII, in providing assistance to any State Party exposed to danger as a result of a violation of the Convention. In addition, international initiatives for the development and application of scientific advances for the global prevention of disease, or for sustainable food supply and security are significant in the implementation of Article X.

17. The nature and breadth of this year’s topic once again emphasises the need to bring in a diverse range of expertise from academia and industry when relevant to assist us in identifying and reviewing significant advances, and in considering their implications for implementation of various aspects of the Convention. In particular, those aspects related to the increasing convergence of chemistry and biology underline the importance of continuing to build and sustain coordination between the BTWC and the Chemical Weapons Convention to assist analysis of the potential benefits and risks resulting from advances in scientific and technological areas of mutual interest.