SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENTS RELEVANT TO THE BIOLOGICAL WEAPONS CONVENTION

Submitted by the United Kingdom

Introduction

1. The Preparatory Committee for the Sixth Review Conference of the Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons (BTWC) decided to ask the Secretariat to prepare a background information document on new scientific and technological developments relevant to the Convention, to be compiled from information submitted by States Parties as well as from information provided by relevant international organisations. In accordance with this decision, the Secretariat has requested States Parties to submit appropriate information, suggesting that submissions be as concise as possible, summarising the major points and providing references to further information.

2. The United Kingdom has provided such papers to all previous Review Conferences to assist in assuring, as mandated by Article XII, that the purposes of the Convention are being realised, taking into account scientific and technological developments relevant to the Convention. These papers considered relevant advances in science, medicine, agriculture and industrial fields and their implications for the scope of the Convention, including the balance between their potential benefits for prophylactic, protective or other peaceful purposes and their potential for misuse.

3. The United Kingdom paper submitted to the Fifth Review Conference in 2001[1] provided a comprehensive and detailed review of the background to and advances in a number of relevant fields, including continuing developments in technologies discussed in submissions to previous Review Conferences, as well as some emerging and rapidly growing fields. The current paper takes a similar broad approach and includes some new areas where recent achievements have potential implications for the Convention, e.g., nanotechnology and synthetic biology. However, less detailed background descriptions of technologies are given here, since these were provided for many of the topics in the previous paper. In addition, the increasing concern of non-government bodies about the potential for misuse of scientific developments, particularly following the anthrax attacks in the United States in 2001, has led to an increase in the number of publications on the subject. These either provide a comprehensive review of a range of relevant scientific and technological developments[2], or focus on individual technologies. Therefore, this paper provides a summary of the major advances under each topic and, where appropriate, references for further information[3].

[1] BWC/CONF.V/4/Add.1

[2] For example, 'Globalization, Biosecurity, and the Future of the Life Sciences' (2006), Committee on Advances in Technology and the Prevention of Their Application to Next Generation Biowarfare Threats, Institute of Medicine *and* National Research Council *of the National Academies.* The National Academies Press, Washington, D.C. www.nap.edu

[3] Individual references are quoted as examples from the often many sources of information available on specific topics. Citation in this paper does not indicate UK endorsement of the views expressed by the authors.

Overview

4. The global spread of scientific and technological advances in the life sciences is driven by increasing international co-operation in research, public health, agriculture and the pharmaceutical and biotechnological industries, and by the increasing global threat of new and emerging diseases. This trend is assisted by continuing developments in information technology, including the internet, which increase the availability of knowledge worldwide. The rapid pace of developments in science and technology relevant to the Convention, and the global spread of related knowledge demonstrate that implementation of the Convention has not hampered the progress of science for the prevention of disease, or for other peaceful purposes. The global spread of technology and knowledge is of great benefit to humanity. However, conversely, the wider dissemination of knowledge and skills in dual-use scientific applications increases the global potential for their misuse in biological weapons by State or non-State actors.

5. Increasing awareness among both government and non-government sectors of the potential for the misuse of the life sciences, including through the work of the BTWC intersessional process, has contributed to the development of biosecurity policies and practices. At the international level, the World Health Organisation (WHO) has included a chapter on laboratory biosecurity in its Laboratory Biosafety Manual, Third Edition (2004), and has recently prepared a more detailed guidance document, 'Biorisk management: Laboratory biosecurity guidance, September 2006'[4]. The 2003 BTWC Meeting of Experts highlighted that some States Parties have introduced national legislation and other measures to establish and maintain security and oversight of dangerous pathogens and toxins. In addition, the 2005 BTWC meetings on codes of conduct for scientists increased outreach to non-government bodies and civil society, raising awareness of the issues and contributing to an increased interest in developing codes of conduct among the scientific community. Such activities have had a positive impact on maintaining the norm against biological and toxin weapons, and contribute to strengthening the Convention.

6. The UK continues to hold the view that the BTWC fully covers all microbial and other biological agents, and toxins, whether naturally occurring or artificially created or altered, as well as their components. This applies to scientific and technological developments included in the following sections of this paper, and in UK papers prepared for previous Review Conferences. Many of the developments included here are further advances and new applications in fields discussed previously, where an already recognised potential for misuse may be sustained or increased. However, new approaches to medical countermeasures and biological detection and identification could effectively decrease the potential for misuse by providing improved defensive measures and thus making the acquisition and use of BW less attractive.

7. The implications of new and rapidly emerging technologies, such as synthetic biology and nanotechnology, are difficult as yet to predict, but these are clearly areas with vast potential for both beneficial and detrimental applications. It will be important to monitor regularly the advances in such technologies and assess their implications for the BTWC. Indeed given the accelerating pace of developments in science and technology in general, the UK continues to

[4] http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf

hold the view, as expressed in its paper for the Fifth Review Conference, that the Review Conference should consider a process of more frequent, perhaps annual, assessments of scientific and technological developments relevant to the BTWC.

Science and technology review

Genomics and proteomics

8. The first bacterial genome sequence was published over 10 years ago, and hundreds of bacterial genome sequences are now publicly available via the internet. Genome sequences for almost all bacterial and viral pathogens of public health importance, including those which might be used as biological weapons or bioterrorism agents, are now available. Much of the effort is now directed at understanding the extent of sequence variation that exists within a species. Thus, large numbers of strains of given pathogens are being sequenced in parallel. Genome diversity is generated by a variety of mechanisms, including mobile genetic elements and bacteriophages. An examination of the 20 *Escherichia coli* genomes sequenced so far illustrates this dramatically, with the genome size ranging from 4.6 to 5.5 million base pairs; much of the variation appears to be of phage origin. These new sequencing projects are made feasible because of technology developments including capillary sequencing machines and high density overlapping oligonucleotide chips which allow the rapid re-sequencing of genomes.

9. There are equally dramatic advances in the sequencing of mammalian genomes. The human genome and mouse sequences have been completed and there are currently several hundred eukaryotic genome sequencing projects underway. Again, the focus is now moving towards understanding the extent of genetic diversity that occurs within a species. The International HapMap Project[5], initiated in 2002, is a multi-country effort to identify and catalogue genetic similarities and differences in human beings. The HapMap is expected to be a key resource for researchers to find genes affecting health, disease, and individual responses to drugs and environmental factors. The project is also addressing potential ethical issues related to examining samples from different ethnic populations. All information generated by the project is made freely available in the public domain.

10. The potential applied benefits of these sequencing projects are profound. In the microbial area they are underpinning work to devise new pre-treatments (such as vaccines) and therapies for disease. They allow new approaches to monitor the spread of disease to be developed, and new approaches to the identification of pathogens to be devised. In the case of human (and mammalian) genome sequencing projects, they allow new genetic tests for disease to be devised and should allow closer matching of drugs to individuals for the maximum therapeutic benefit with minimal side effects.

11. The potential for misuse is also apparent. For example, the laboratory synthesis of a viral genome (poliovirus) has been reported, opening the possibility that at least some agents (or chimaeric agents) could be constructed *de novo*. Similarly, it is possible that information on the diversity of the human population could be exploited to target specific groups with harmful agents.

[5] www.hapmap.org

12. The field of proteomics also continues to develop rapidly. The proteome is traditionally studied through a combination of gel electrophoresis and mass spectrometry. New methods have concentrated on: developing high throughput and automated technologies for rapid and repeatable analysis of multiple samples; sample fractionation strategies for looking at specific sub-groups of proteins; and development of ever more sensitive and powerful mass spectrometers for detecting low levels of proteins. Other developments include progress in non-gel techniques for separating and analysing protein mixtures.

13. One rapidly expanding area is comparative proteomics. Proteins from different growth conditions, strains, or species can be labelled so that differences can be detected by mass spectrometry. This technique is being used to identify proteins that have a role in virulence, interaction with the host or the environment, and antibiotic resistance. Identified proteins can be used as vaccine candidates or targets for therapeutics or diagnosis. In addition, identifying proteins expressed under a wide variety of conditions can lead to the identification of targets for detection systems.

14. Another area of development is the study of the 'immunome', the immunodominant proteins of an organism. Studying the immune response to infection and the interaction of antibodies and antigens can lead to an understanding of the humoral immune response and identification of antigenic determinants for inclusion in future vaccines. The introduction of protein arrays (a combination of microarray and proteomics technologies) enables the rapid analysis of hundreds of proteins in parallel. These techniques are being used to identify detection and diagnostic biomarkers as well as possible vaccine candidates. Future advances are likely to include methods to analyse and identify proteins involved in the cell mediated response.

15. Proteomics can be used to differentiate isolates or strains and greatly enhances our knowledge of host-pathogen interactions, protein-protein interactions, host response to infection, and pathogenesis. Proteomics can be used to identify candidates for diagnosis, therapy, detection systems and vaccines. Equally, the knowledge gained from proteomics could be exploited for non-peaceful purposes.

16. The generation of biological data continues to increase due to such techniques as genome sequencing, proteomics and high throughput data-collecting. In order to interpret and utilise this data, the field of bioinformatics has also continued to develop in parallel, supported by advances in computer technology and the accessibility of data and tools via the internet and on computers. As bioinformatics has developed, several new disciplines have arisen which are collectively known as the 'omics'. As well as genomics and proteomics, the newer topics include metagenomics (the genomic analysis of microbial communities in the environment), transcriptomics (the study of all mRNA produced under a given set of environmental conditions), epigenomics (a study of DNA methylation patterns which exhibit different specific profiles under normal and diseased states) and metabolomics (a study of the production of metabolites under specific conditions). There is also a move away from the study of individual elements and towards the systems biology approach, which seeks to integrate biological data in an attempt to understand the complex interactions of genes, proteins and cell elements.

17. Bioinformatics can be used in the analysis and modelling of pathogens. It can assist in the understanding of pathogenicity and virulence, and in how pathogens adapt to and overcome host immune systems and different antibiotics. It can also provide a deeper understanding of the host biology, and thus a greater understanding of the processes and consequences of host- pathogen interactions. As the applications and tools are developed and refined, bioinformatics will be able to produce predictions for vaccine candidates, virulence factors, drug targets and novel therapeutics with even greater success. However, these techniques could also be misused, e.g., in the development of pathogen strains with increased virulence or drug resistance, or with improved stability to assist survival within the environment.

Synthetic biology

18. The emerging discipline of synthetic biology can be described in simple terms as the process of producing a biological material from its raw physical/chemical elements rather than through natural means. In most cases (an exception is the RNA viruses), DNA acts as a blueprint producing all the essential elements required for a functional biological system. Massive technological leaps in the field of chemical DNA synthesis over the past 5-10 years have facilitated the rapid progress of this technology. In particular, the synthesis of increasingly longer DNA constructs has made *de novo* synthesis of some viruses a reality, though the technology is not yet capable of effective chemical synthesis of bacterial genomes. As a consequence of these developments, there has been a great influx of commercial companies worldwide that now offer various services relating to DNA production. It is commonplace for companies to offer synthesis of sequences of 10000-40000 base pairs; for comparison, the polio virus has a genome of 7500 base pairs.

19. In combination with an appropriate recombinant expression system, there is the potential for significant quantities of a protein to be produced from synthetic DNA. Expression systems have been developed and are commercially available for production of proteins within bacteria, yeast, plants, filamentous fungi, insect and mammalian cells. In the case of synthetic DNA that encodes viral genomes, techniques have been developed to allow the *in vitro* assembly of infectious viral particles from the synthetic construct.

20. A more ambitious aspiration in the field of synthetic biology is the construction of functional genetic circuits in cells and microorganisms, merging engineering approaches with biology. The ultimate aim is to develop a set of standardised genetic building blocks with known characteristics that can be drawn upon to assemble circuits with practical applications, such as detection of toxic chemicals, explosives and biological agents, diagnosis of disease and therapeutic intervention, production of pharmaceuticals, bioremediation of pollutants, and energy generation.

21. The potential benefits from developments in synthetic biology are wide ranging. The significant technological advances in chemical DNA synthesis allow an alternative and possibly more efficient and cost-effective means of production of natural products with potential uses in therapeutics or for other beneficial purposes. A particular benefit, especially when analysing proteins from dangerous organisms, is that the gene can be chemically synthesised, then cloned into, expressed in and purified from a suitable host cell without the need to handle the original organism. This may preclude the need for containment facilities and procedures normally

required for work on pathogens. Use of DNA synthesis technology for rapid, efficient synthesis of viral, and potentially other pathogen, genomes may facilitate the study of pathogenicity and virulence, and the development of vaccines and therapeutics. Recent publications on the reconstruction of an infectious poliovirus and of the 1918 Spanish influenza virus from genetic sequence data using chemical synthesis suggested such potential benefits from the work. However, this technology also has the potential for misuse, and indeed such concerns were raised in reactions to the work described on polio and influenza virus reconstruction.[6]

22. Production of virus from synthetic DNA requires many extra co-factors, as well as a significant technical/scientific knowledge and expertise. Coupled to this is the fact that only small viral genomes can currently be synthesised as an intact sequence. Larger genomes such as those found within the smallpox virus or within bacterial pathogens cannot currently be made. As a cautionary note, one might suspect that where technology exists as the only barrier to something being achievable it is only a matter of time before that barrier is overcome. Recently, concerns have been raised about the capabilities of commercial companies to supply increasingly longer chemically synthesised DNA segments. The ready commercial availability of custom DNA sequences, made to order, means that it is now more than just theoretically possible for someone, with the expertise and money, to reconstruct pathogenic viruses, or even design new viruses with properties that would be suitable for hostile use. Concerns have included the fact that such activities are largely unregulated; however, the vast array of contexts and technologies within which synthesised DNA sequences are used would make it difficult to police their supply. Nonetheless, some commercial suppliers use specific software to screen all sequences ordered for pathogenic DNA sequences before proceeding with orders.[7]

23. It is recognised that the rapidly developing and growing field of synthetic biology also has the potential to create risks for society, due to either unintentional harmful consequences for health or the environment, or to deliberate misuse. There have been calls for the community involved in this field to examine the implications at this relatively early stage of development, and ensure that the benefits balance the risks.[8]

Delivery of bioactive molecules

24. *RNA interference (RNAi).* RNAi has emerged as a promising technology for the development of therapeutic approaches to a number of diseases, and as a research tool in functional genomics. Small interfering RNA (siRNA) molecules were initially identified in plants but also function in mammalian cells. They are able to promote the sequence-specific degradation of messenger RNAs and are therefore able to suppress gene expression.[9] The effects are potent. This approach has been widely used in research for the suppression of specific genes

[6] Details of this work and the reactions to it are described in several publications and articles; one example is : Wimmer, E. (2006) The test-tube synthesis of a chemical called poliovirus. *EMBO reports* VOL 7 Special Issue: Science & Society.

[7] Peter Aldhous (2005) 'The bioweapon is in the post' New Scientist 2525, p8.
From issue 2525 of New Scientist magazine, 09 November 2005, page 8

[8] For example, see: Nature Vol 438, p423 (2005). Let us go forth and safely multiply. Commentary by George Church,; Jonathan B. Tucker and Raymond A. Zilinskas, "The Promise and Perils of Synthetic Biology," The New Atlantis, Number 12, Spring 2006, pp. 25-45; www.syntheticbiology.org.

[9] Background information to this technology can be found in many published reviews and articles, such as: Couzin J. 2002. Breakthrough of the year: small RNAs make big splash. Science 298, 2296-2297.

in cell culture and in the generation of genetically manipulated animals. The object of such experiments has been to demonstrate gene function. However siRNA are sufficiently active to function as potent antiviral agents and offer the possibility of modulating gene expression in adult animals. The effectiveness of siRNA as antivirals has been demonstrated, to proof of principle, in a number of *in vivo* systems, e.g., Ebola virus infection of primates. However, the major challenge to the widespread use of this technology continues to be the development of a suitable delivery system. There is intense research activity in this area towards development of clinically useful, licensed antivirals and gene therapy applications.

25. For therapeutics, the delivery systems must be minimally invasive and safe. Delivery of siRNA by viral vectors, e.g., lentivirus or retrovirus vectors, could lead theoretically to the highly efficient silencing of a given gene in adults. Choosing the appropriate vector may even lead to germ line transmission. However, such vectors would currently not be considered safe for human use and may have long-term oncogenic effects.

26. As with many other developing technologies, RNAi has the potential for misuse as well as for benefit. Theoretically, the technology now exists for the long-term, efficient silencing of an allele that segregates with ethnicity. Alternatively, silencing of genes that function in, e.g., innate immunity could lead to conditions that mimic, at least superficially, natural disease. This may represent a significant change in the utility of genetic weapons technology. The current issues relating to safe delivery of siRNA in human therapeutics may not be considered a significant hurdle to potential misuse for hostile purposes.

27. *Gene therapy.* Gene therapy still offers major potential benefits for human and animal medicine in the treatment of genetic diseases and in the modulation of gene expression for a variety of other reasons, e.g., suppression of inappropriate immune reactions. However, there have been set-backs in the development of vectors for delivery of such therapy: for example, repeated application of high doses of adenovirus vectors may lead to immunopathology and lentivirus/retrovirus vectors may be associated with oncogenic effects. However there is a variety of possible vectors and steady progress continues to be made in their development and use in both humans and animals. Achieving sufficient efficiency of gene expression remains a major challenge that currently appears to require a viral vector system rather than synthetic systems such as liposomes.

28. Safety considerations notwithstanding, lentivirus/retrovirus vectors would also have the potential for misuse, providing the opportunity to achieve long-term expression of a deleterious gene in a target population. However, the technology does not exist currently to reliably target such a foreign gene to individuals possessing, e.g., a DNA sequence associated with a particular ethnic origin. In contrast, the silencing of alleles associated with ethnicity (or that segregate in any group of individuals, due to a limited opportunity for interbreeding) now appears theoretically possible.

Protein expression and production technologies

29. While upstream technologies for protein production have made significant progress, particularly in the research laboratory, there has been a gradual consolidation of related downstream processing technology. Relative to fields such as gene therapy, protein production is

a more mature area, particularly on a large scale. The biopharmaceutical industry is highly regulated and adoption of new processes is generally slow and subject to rigorous testing and review due to the high cost of obtaining regulatory approval. The entry cost for those producing products such as vaccines and therapeutic agents for human use is significant and the large number of small scale developments in the research laboratory continue to find only limited application on an industrial scale.

30. Continued progress in recombinant DNA technology has seen the development of techniques to allow cloning directly into expression vectors and the high fidelity, easy transfer of genes between expression vectors. This decreases the time required to clone a target gene and reduces the level of expertise required. There have also been developments in vectors and plasmids to enhance the expression of soluble and problematic proteins. These developments have resulted in the relatively easy expression of increasing number of proteins that were previously difficult to express, or to express in a form suitable for purification.

31. This progress in recombinant technology has led to an increasing focus on the production of proteins from pathogenic hosts in micro-organisms which are more easily and safely handled on a large scale. Such programmes, however, remain costly and require high containment facilities and skilled labour. Even in the vaccine field, where there are strong commercial incentives, progress remains slow, as shown by the drive in many countries to produce a recombinant protective antigen as the basis of a new generation anthrax vaccine. After several years, this is still not yet a major commercial reality.

32. In the purification field, there has been an increased emphasis on the development of affinity media for both laboratory and manufacturing scale purification of affinity tagged protein. Such methods result in the production of high yields of specific product; since the tag is small, the protein's activity is unaffected so there is no need to remove it following purification. New manufacturing media have been developed that allow the recovery of secreted protein direct from crude fermenter feedstock without the need for centrifugation. The range of protein purification platforms available has continued to develop over the past five years. New systems are available for small scale (between laboratory and manufacturing scale) purification of protein. Systems are also now available for laboratory scale automated purification of several proteins at a single time.

33. Continuing progress in processing equipment for the pharmaceutical and biotechnology industries has included the development of disposable equipment for operations traditionally performed in stainless-steel tanks and piping. A key example is the emergence of portable bioreactors featuring disposable contact materials that eliminate cleaning, sterilisation and validation. Cell culture is performed in plastic bags filled with media and incubated on a rocking platform; these bioreactors provide a closed system and are delivered, with all fittings and filters, sterile and ready for use. Suggested applications include virus production, monoclonal antibody production, insect cell culture and production of human therapeutics. Currently available equipment has been scaled up to over 500 litres culture volume, but is also available in various sizes from 1 litre.

34. Past proposals to utilise modern technology to express proteins of interest in transgenic high volume production vehicles, such as bovine milk, have been slow to come to fruition. Similarly, agricultural adaptation of life science technologies to produce vaccine antigens or

other bioactive molecules in transgenic plants has not yet become popular. These methods are seen as potentially high volume, low cost alternatives, and may be attractive to developing countries with limited biopharmaceutical facilities. Although a popular idea in the mid-late 1990s, and despite significant research efforts, these technologies have not attracted the levels of funding required to turn the research into a viable means of general, large scale production in the manufacturing industries. Moreover, the long lead times and high regulatory costs associated with them have meant that licensed applications are uncommon and this is likely to remain the case in the immediate future.

35. These developments obviously have potential for misuse. More successful protein expression and production strategies, including portable systems, may facilitate processes for potential BW production as well as providing benefits to the pharmaceutical and biotechnology industries. For example, it would be possible for transgenic plants to be engineered to produce large quantities of bio-regulatory proteins or toxins, which could be extracted from plant cells or used directly as BW agents. Use of transgenic plants would eliminate the need for much of the sophisticated equipment normally required and could potentially provide a covert means for producing large amounts of product. However, while the potential for misuse of protein production technology remains, the creation of effective biological weapons does not require a highly purified product to be a serious threat. Conversely, the preparation of effective protective material does require significant purification and any associated technologies could be applied to the development of prophylactic products against biological weapons.

Infectious disease

36. Much information on national and international mechanisms for infectious disease surveillance and control was exchanged at the BTWC Meetings in 2004. States Parties agreed on the importance of, *inter alia,* improving national and regional capabilities and supporting and strengthening existing networks of relevant international organisations.[10]

37. *Human infectious disease patterns and emerging diseases.* Infectious disease patterns fluctuate on a seasonal, annual or seemingly random basis, which can often be attributed to, e.g., changes in local and atmospheric weather patterns, migration of animals and humans, changes in agricultural practice, internal displacement of people, importation of animals and foodstuffs through global trade, or as a result of conflict. Over longer timescales, global climate change has also been predicted to influence future disease patterns. Our ability to understand these driving forces can aid our assessment of the significance of these disease patterns as global and natural emerging disease issues. At a local, national and global level there are various surveillance systems that record the incidence of infectious disease (including syndromic surveillance) or scan the newswires for the latest information on the disease outbreaks. Such information is gathered and disseminated to interested organisations and parties through various global disease outbreak reporting systems such as GPHIN (Global Public Health Intelligence Network) or MEDISYS (EU initiative). This and similar information from regional organisations and national government reports is assimilated as e-mail updates provided by WHO and ProMED (Programme for Monitoring Emerging Diseases). Indeed, the emergence of SARS and avian

[10] BWC/MSP/2004/3

influenza was brought to the fore by these reporting systems, and they continue to keep the global public health community in touch with new and ongoing events. This information forms one very important basis of our understanding of global disease; it helps inform our strategies for national emerging disease control, and provides an early warning of emerging or unusual events, including those potentially of biodefence concern. It also informs subsequent hazard identification, risk assessment and risk management activities.

38. Hazard identification aims to pre-empt diseases that might emerge through either natural or unnatural means. Through our knowledge of global infections, likely scenarios can be identified, possible risks can be highlighted and the prioritisation of target issues can be determined through understanding disease ecology (such as vectors, reservoir hosts, abiotic risk factors) and epidemiology (such as attack rates, case fatality rate, susceptible groups, hospitalisation rates), and their possible economic and public health burden. Having identified a likely hazard, modelling strategies are increasingly being used as part of the risk assessment process. These can be used to investigate and better understand the potential public health burdens arising from the possible spatial and temporal spread of existing, new and emerging infections and the impacts of interventions to control them. As a consequence of applying risk assessment processes, which are usually iterative as greater understanding is developed, policy makers are better able to develop strategies for risk management that inform contingency plans, and the need for, and location of, stockpiles of drugs and vaccines, for example. Channels of communication, and national and international collaborative networks are also being increasingly established, and at a local, regional, national and international level, emergency preparedness and response units are able to be better briefed and trained on likely scenarios, through the use of computer hardware, surveillance data, and toolboxes of models and mapping software.

39. The ability to respond to and prepare for existing diseases can be demanding. Preparing for the unknown, such as a new emerging disease or a bioterrorist release, can be much more testing of national and global resilience and challenge networks, infrastructures and public health plans. Robust internationally networked surveillance, assessment and management systems and strategies are a key factor in helping to mitigate the effects of such an incident.

40. *Pathogenicity and virulence.* Efforts to better understand the pathogenicity and virulence factors contained in the genetic make-up of microorganisms has driven the vast number of microbial genome sequencing programmes. Whilst this has greatly improved the knowledge of how many microorganisms effect pathogenesis through characterisation of their discovered virulence factors, there is an increasing awareness that many of these virulence factors act in concert with one another and with host factors.

41. To this end, there has been an increase in the study of the host response to infection in order to fully characterise the virulence of microorganisms. Although DNA microarrays have been used to study the gene expression profiles of pathogens, there has recently been a substantial increase in transcriptional profiling of the host in response to infections with pathogens. Experiments that measure the host transcriptional response to a pathogenic strain, relative to an attenuated strain that lacks a key virulence determinant, provide knowledge on how these interactions develop into pathogenesis. For example, a reduced response to a virulent strain suggests an inhibition of the immune response, whereas elevated expression levels might point to the stimulation of an excessive or inappropriate immune response. The trend towards these types

of global approaches to understanding pathogenicity and virulence will continue to increase as standards and techniques become refined and costs reduce.

42. This increase in knowledge in the host's role in pathogenesis and, consequently, an understanding of mechanisms of immune protection may potentially open an avenue for misuse through the development of molecules for reducing the effectiveness of the immune response to specific pathogens.

43. *Vaccines and therapies.* Over the past 5 years there has been a greater interest in the development of vaccines for a wide range of purposes. Because of increased levels of antibiotic resistance, vaccines are increasingly favoured for medical countermeasures against infectious disease in the civilian community. In addition there has been a major investment in the funding of vaccines for biodefence. Vaccines for the prevention or treatment of diseases such as cancer are also being developed. Major advances in pathogen vaccine research and development continue to be facilitated by the availability of genome sequences of pathogens, high-throughput screening methods and the applications of bioinformatics. Such approaches mean that vaccine discovery continues to have the potential to proceed much faster and with a better chance of leading to acceptable medical countermeasures.

44. Progress in the areas of vaccine development highlighted in the UK paper submitted to the Fifth Review Conference has continued. Research continues to focus on the development of recombinant sub-unit vaccines, and DNA vaccine technology has also advanced, with many publications and clinical trials taking place for a range of diseases, such as HIV, hepatitis B and malaria. Recently, development of DNA vaccines to protect against influenza viruses has progressed to the stage of early clinical trials. Although some DNA vaccines have now been licensed for veterinary use, e.g., to protect against West Nile virus in horses, there are as yet no human vaccines licensed, or in later phase clinical trials. The current challenge is to bridge the gap between research and commercial development and manufacture of DNA vaccines.

45. The development of more effective vaccine delivery systems continues, for example, through improved adjuvants, live vectors and microencapsulation technologies. In particular, in the field of inhalation delivery, a variety of new aerosol generating devices have been developed commercially for the more effective delivery of therapeutics.

46. Progress has continued in the development of anti-viral drugs. Several drugs reported to be effective against poxvirus infections, including cidofovir and SigaST246, are currently being evaluated. However, as with many antiviral drugs it is possible that the side effects of these drugs will preclude their prophylactic use. There has also been a resurgent interest in antibodies for the prevention and treatment of disease, and monoclonal or polyclonal antibodies which are effective against viruses such as poxvirus and Venezuelan equine encephalitis virus have been reported.

47. A number of other approaches to antimicrobial therapy are being explored, including immunomodulators such as cytokines and non-specific immunostimulators. An example of the latter is CpG DNA which is able to activate the innate immune system and provide low level protection against a range of pathogens, including BW agents. However, the only drug of this type with a proven track record continues to be interferon-alpha, which is used in HIV-AIDS therapy.

48. The potential benefits of advances in this area are vast, but the potential for misuse is also apparent. For example, technologies resulting in improved delivery of vaccines and therapies could also be applied to improved delivery systems for BW agents. Information on immunomodulators and immunostimulators has the potential to be misused for malign attack of the immune system.[11]

49. *Global initiatives.* Information on recent global initiatives to address infectious diseases affecting humans, animals and plants was provided at the 2004 BTWC Meeting of Experts. Such activities have continued and expanded, including initiatives involving collaboration across the human, animal and plant health communities, e.g., the recently launched global early warning system for animal diseases transmissible to humans. The Global Early Warning and Response System (GLEWS) is the first joint early warning and response system created with the aim of predicting and responding worldwide to animal diseases, including zoonoses. The system builds on the added value of combining and coordinating the tracking, verification and alert mechanisms of OIE, FAO and WHO, and will allow control measures that will benefit both animal and public health.

50. In May 2005, The WHO World Health Assembly approved the revised set of International Health Regulations, the purpose of which is to ensure maximum protection of people against the international spread of diseases, while minimising interference with world travel and trade. Countries now have much broader obligations to build national capacity for routine preventative measures, as well as to detect and respond to public health emergencies of international concern. These routine measures include public health actions at ports, airports and land borders, and for means of international transport.

Toxins and other bioactive molecules

51. Structural studies involving microbial and animal toxins remain an area of interest for research groups worldwide. Much of this work is required for the development of vaccines or anti-toxins, in particular, against specific venoms. Certain animal toxins, such as those found within venoms, are highly complex in structure and are traditionally thought of as being difficult to produce via recombinant systems. However, there are now a number of products commercially available that are specifically designed to aid protein folding, suggesting that these technical barriers may no longer be an obstacle.

52. The therapeutic potentials of the A-B subunit toxins, such as botulinum toxin, continue to be explored. The catalytically active and toxic A-subunit portion of these toxins can be conjugated to antibodies raised against specific antigens found on the surface of tumour cells. The immunotoxin subsequently created has the potential to act as a form of site-directed anti-cancer therapy, killing the tumour cell upon binding. Also, the specific nature of some B-subunits for particular cell types is being studied for its potential exploitation in intracellular delivery mechanisms. For example, the B-subunit of tetanus toxin is being considered for its ability to deliver therapeutic agents to neural cells for the treatment of neural dysfunctions.

[11] See, for example, Nixdorff K. (2005) 'Assault on the immune system' in Disarmament Forum. Science, Technology and the CBW Regimes. 1, 25-35

53. Improved methods for production of recombinant animal toxins may result in a capability to produce significant quantities of toxins that are difficult to isolate from a natural source. This has obvious implications for the possible use of such toxins as BW agents. Information gained in studies on the utility of toxin subunits in targeting therapeutic agents to specific cells also has the potential to be exploited for targeting harmful agents.

54. Therapeutic use of bioactive molecules, particularly peptides, continues to be a major interest for the pharmaceutical industry. In general, biologically active peptides are highly specific for a particular cell surface receptor and bring about an intracellular effect on a target cell and/or a physiological change in the target organism. Commercial peptide synthesis has revolutionised research in this field. Many companies now offer peptide synthesis services ranging from small scale synthesis at the milligram scale for laboratory use, up to the hundred of kilograms scale produced to Good Manufacturing Practice standards. Alternatively, significant quantities might be produced in recombinant microroganisms, or in transgenic plants or animals.

55. A recent example of a licensed bioactive peptide is a synthetic conotoxin compound used in the treatment of severe chronic pain. It is anticipated that other compounds produced by cone snails may also have therapeutic applications. The use of phage/ribosomal display technologies, combinatorial chemistry, molecular modelling and high throughput screens will facilitate the discovery and design of potential therapeutic peptides. Such methods could also result in the intentional or unexpected discovery of compounds with potential for misuse.

56. Delivery of sufficient quantities to the appropriate target cells or tissues is a significant challenge to the development of therapeutic peptides, with delivery across the blood/brain barrier, for example, remaining a significant problem. Difficulties in delivering bioactive molecules would also affect the utility of such compounds as BW agents.

Detection and identification technologies

57. Improved methods in rapid DNA detection using polymerase chain reaction (PCR) analysis have been developed. These include a greater range of fluorescent detection molecules and more rapid methods for PCR amplification. There are now a number of commercially available instruments capable of use in the field environment. At least one commercial system allows single shot sample preparation and PCR amplification. A number of gene probe systems for array and other format detection have been developed along with strategies to overcome problems of non-specific hybridisation. Hand held devices are commercially available with utility for rapid diagnosis and environmental sampling. A number of different methods for micro-arraying are now available.

58. Antibody based technologies have advanced considerably with a number of commercial systems being available. Many operate in batch mode and require a secondary antibody and label step to achieve detection by reporters such as fluorescence or luminescence. The requirement for secondary reagents increases the logistic burden of these systems for field operation. However, the use of freeze dried or lyophilised reagents has improved the operability of these instruments. The UK military employs two antibody based biosensors in its Integrated Biological Detection System for environmental monitoring.

59. Advances in antibody based production technologies have matured with hybridoma cell lines being employed as a standard technique for monoclonal IgG antibody production. Advances in phage display technologies have allowed for the production of single-chain antibody systems which are more robust than conventional antibody systems. An emerging area is the use of more robust antibody systems that are developed in animals such as camels and sharks. These offer the potential for more robust antibodies capable of operating in harsher environments and over greater temperature ranges than conventional antibodies.

60. Advances have been made in non antibody based recognition, with DNA aptamers being developed to recognise a number of different antigens. Oligosaccharide and other carbohydrate chemistries have also been shown to recognise some biological agents, and array-based chips for carbohydrate detection are now commercially available for non-defence applications. Aptamer systems that incorporate nanoparticle quantum dots have been developed for fluorescent resonance energy transfer (FRET) reporting of the presence of antigen. A similar technology based on gold nanoparticle detection of biological reagent, where colour change is used as an indicator of the presence of toxins, has also been recently reported.

61. Advances in optical biosensors have led to the development of a number of commercially available systems for rapid on-line direct detection and identification in the field, including significant development of miniaturised systems. Many of these instruments are based on evanescent wave detection such as surface plasmon resonance. These systems have been demonstrated in the field as capable of near real time detection and identification of airborne antigen. Many of these systems require a secondary reporting system to allow detection of the larger antigens such as bacteria and viruses. Techniques such as light scattering surface plasmon resonance and metal clad leaky waveguide have been developed to directly detect these analytes.

62. Techniques that concentrate particulates using ultra sound, electro- and dielectro - phoresis are being developed to improve the limit of detection for biosensor systems. The use of high frequency ultra sound has also demonstrated that it is possible to improve detection limits of antibody based systems through in-line cell disruption.

63. Bioluminescence techniques have been developed for generic detection, and a commercial company now produces recombinant thermal stable luciferase. A specific detection instrument based on bioluminescence using adenylate kinase has been designed, produced and tested in the laboratory. Low limits of detection of bacteria have been demonstrated. These technologies remain reagent intensive and it is hoped that advances in the auto-fluorescent detection of aerosol particles will have greater utility in the field.

64. Dip stick technologies based on lateral flow devices offer a simple and easy-to-use method of detection and identification. There have been a number of advances in the area and many systems are now commercially available. These systems offer detection of a number of analytes and, whilst many still use gold sol or coloured bead systems as reporter, a number now offer different labels for automatic readout. Carousel type systems have also been developed for multiple assays.

65. Recent years have thus seen continued advances in applications of a wide range of principles that increase the utility of field systems for biological detection. Such technologies have applications in medical, agricultural and other fields, as well as in biological defence.

Nanotechnology

66. Nanotechnologies can be defined as 'the design, characterisation, production and application of structures, devices and systems by controlling shape and size at nanometre scale'. Such technologies are widely seen as having great potential to bring benefits to many areas of research and application, and are attracting rapidly increasing investments in many parts of the world. However it is recognised that the rapid expansion of this field may raise new challenges in the safety, regulatory and ethical domains that will require wider debate.[12] Consequently, stakeholders are engaging to ensure the responsible development of these emerging technologies. The European Commission has published a Nanotechnology Action Plan, and the OECD is also working to promote international cooperation in the health and environmental safety related aspects of manufactured nanomaterials. Various national, regional and international stakeholders and initiatives interact with each other, contributing to the development of controls to manage the risks posed by nanotechnologies.

67. Applications in nanobiotechnology and medicine show particular promise, and areas such as disease diagnosis, drug delivery, and molecular imaging are being investigated intensively. Nanocrystalline silver, which is known to have antimicrobial properties, is being used in wound dressings in the US.

68. Work on biological detection, using a variety of means involving nanoscale materials and their manipulation, is expected to result in new options for more sensitive, easy to use and cheaper detectors. There may also be an indirect impact through development of increasingly autonomous systems and novel (including biological) power sources involving nanoscale technology. Applications in drug delivery might include, for example, nanoparticles capable of targeting specific diseased cells, containing both therapeutic agents and a sensor that regulates their release into the cell. Gene therapy, where the DNA has been packaged into nanometre-scale particles is also an option. A more direct approach to drug discovery may become feasible using nanotechnology techniques to study drug-receptor interactions at the single molecule level. Other applications are leading to the production of materials and devices such as scaffolds for cell and tissue engineering, and sensors that can be used for monitoring aspects of human health. Further details of these and other potential applications are given in the referenced report.

69. Some emerging technologies in this field have potential for misuse, e.g., in the development of novel or enhanced biological agents or improved delivery methods. However, it is difficult to predict the outcome of many research areas and thus the impact on potential BW applications. Clearly, this rapidly evolving area is one to watch for emerging implications.

[12] 'Nanoscience and nanotechnologies: opportunities and uncertainties.' The Royal Society and the Royal Academy of Engineering. July 2004. RS Policy document 19/04. ISBN 0 85403 604 0. www.royalsoc.ac.uk; www.raeng.org.uk

Developments in agriculture

70. *Animal disease.* 2006 marks the five year anniversary of the last foot-and-mouth (FMD) disease outbreak in the UK, and in light of the evolving global spread and heightened interest of the risk of Avian Influenza (AI), the UK government, as part of its overarching veterinary surveillance strategy, continues to develop policies and operational readiness necessary for controlling and managing an outbreak of exotic animal disease. This includes:

(i) Strengthening the scientific modelling and evidence capability to assess risk and support disease control policies;

(ii) Strengthening links at national and local levels with a wide range of stakeholders and operational partners. For example, in 2004 a multi-agency and cross-disciplinary group with representation from veterinary and public health bodies was set up to identify and discuss infections with potential for zoonotic transfer;

(iii) Development of a national contingency plan for the main exotic diseases that provides the framework for national and local operational and veterinary instructions. These plans have been tested in simulation exercises for FMD (2004) and AI (2006), and provide valuable information that ensures the UK is in a current state of readiness to tackle future national outbreaks of these diseases. Further contingency plans are being prepared for other OIE listed exotic diseases. Standardisation of laboratory techniques between veterinary and public health authorities for detection of zoonotic diseases such as AI, West Nile Fever (WN) and salmonellosis has facilitated epidemiological investigations and control of outbreaks.

71. Apart from the early detection and management of exotic animal diseases, the veterinary surveillance strategy aims to detect the appearance of new diseases, particularly potential zoonoses, and detect changes in the occurrence or effects of endemic diseases. A national veterinary sentinel network with strong links to public health authorities has been developed, with an information management system designed to highlight the risks and distribution of veterinary threats to public and animal health.

72. The magnitude and effects of recent national and international animal and zoonotic disease outbreaks such as FMD, SARS and AI and heightened public awareness could make such agents potentially attractive for hostile use. Whilst the above initiatives do not specifically address the deliberate release of agents, they do provide the UK with enhanced surveillance and control procedures that will safeguard animal and public health from disease resulting from natural, accidental or deliberate release of biological agents into the environment.

73. *Plant pests and diseases.* Climate change and human population growth are expected to induce increased pest and disease problems, particularly due to invasive organisms. Pests and diseases are expected to become generally more abundant as temperatures increase. Expanded ranges and increased establishment potential are predicted for a number of existing threats, such as ‘sudden oak death’, Colorado beetle and the western corn rootworm. Increased summer temperatures are expected to lead to the expansion of certain crops, which are marginal in the UK at present (e.g. Soya bean, sunflower and maize). Drier summers and longer growing seasons

could result in the planting of more novel crops. In addition, agricultural diversification could result in the production of a range of novel crops, for example with medicinal uses or for the production of fuel. These crops could bring with them a whole range of new pest and disease problems. Increasing global trade is also predicted to increase opportunities for introductions and establishment in new areas where suitable environmental conditions exist.

74. A few recent examples of pests and diseases new to Britain include potato ring rot, western corn rootworm, 'sudden oak death', the citrus long horned beetle and Chrysanthemum stem necrosis virus. *Thrips palmi* continues to spread in tropical and subtropical regions but still remains largely absent from Europe. Although previous outbreaks in Europe have been successfully eradicated, there are continuing interceptions in Europe on cut flowers, fruit and vegetables, and contingency plans have been updated.

75. Climate changes could also have significant effects on key indigenous diseases such as *Septoria tritici* on wheat. Annual levels of *S. tritici* continue to be moderate to high despite the use of fungicide applications to control the disease. Warmer and wetter weather conditions have resulted in increased multiplication of the disease, and warmer winters are likely to lead to greater over-wintering of this pathogen (and others) and earlier appearance of symptoms in the crop in the spring. Resistance to the major fungicide groups used to combat the disease is now becoming evident, and although resistant varieties can be used, these can decline in effectiveness. Similar patterns have been seen with other indigenous diseases.

76. Despite the best efforts of plant health authorities in putting in place effective control mechanisms and contingency plans, it is expected, on the basis of past precedents, that some alien pests and diseases will become established. Forecasting how impacts will be affected by changing climates, markets, trade patterns, transport methods, etc. is extremely challenging, but detailed studies of emerging problems can provide useful insights. For example, the impact of *Phytophthora ramorum* (sudden oak death) may increase with the emergence of more aggressive strains or through climatic changes producing more optimal conditions for the pathogen. There is concern that different strains of *Phytophthora infestans* (potato blight) could hybridise and produce more persistent strains. In contrast, some presently occurring pests and diseases could potentially become less serious if hotter, drier summers increase or other climatic conditions change which are detrimental to their survival or multiplication.

77. In future, detection and diagnosis of quarantine pests and diseases is expected to rely much less on the 'eye of the inspector' and more on technological tools. The main developments are expected to be in detection within an entire consignment, for example within containerised imports. There is scope to use artificial 'noses' and biosensors to replace sniffer dogs. The advantage of such techniques is the decreased reliance on time-consuming sampling of commodities such as potato tubers. Artificial noses can detect volatiles produced by fungal and bacterial diseases and the technology is being developed for potato store management. Biosensors also have the potential for detection in aqueous environments and in the soil.

78. Techniques for eradicating invasive alien pests and diseases will benefit greatly from new high-resolution landscape datasets. The exploitation of new mobile computing, GPS, digital photography and telephone technologies will greatly enhance the management of pest outbreaks, enabling the rapid transmission of key data between the field, laboratory and headquarters.

79. *Biological pest control.* Scientific advances in molecular biology already have the potential to revolutionise the efficacy of biocontrol agents. However, although the current trend towards an increased use of biological and integrated methods in pest control will increase, it is unlikely that biopesticides will replace chemical crop protection altogether. Whilst there is also a vast source of largely untapped naturally occurring organisms with the potential to provide new toxins suitable for pest control, problems involving formulation, speed of action and efficacy will continue to limit their development. The availability of novel synthetic chemicals with a more benign environmental and health profile will also be a factor.

80. Much research and development continues to focus on *Bacillus thuringiensis* (Bt), most notably in the worldwide growth of transgenic Bt crops. Research aimed at finding new genes and toxins from Bt strains also continues apace worldwide, looking for more effective pesticidal toxins, which potentially increase the range of targets that can be controlled using either conventional biopesticides or transgenic methods. The enhancement of the insecticidal toxicity of Bt toxins is also being achieved by molecular methods. More powerful bioinsecticides have been achieved by combining the attributes of the Bt toxin with other micro-organisms, including baculovirus based systems and other bacteria. The delivery of toxins via other microbial expression systems is a possible alternative to the production of transgenic plants.

81. The search for toxins with potential pesticidal properties has widened beyond *Bt*. For example, the complete genome sequence on the entomopathogenic bacterium, *Photorhabdus luminescens*, which is symbiotic with certain soil nematodes, was published in 2003 and its toxin has been successfully engineered into plants. It is hoped that this organism will be the source of new genes for insect control, perhaps replacing, or supplementing, those of Bt. An unusual soil-dwelling bacterium (*Pseudomonas entomophila*), which is unique in that it is resistant to the immune defences of insects, promises to deliver new biopesticides; the complete genome sequence was published recently. Also, a novel strain of a new species of bacteria, *Bacillus nematocida,* has been discovered with activity against nematodes. Further information on the search for useful natural products can be found in recent reviews.[13]

82. Products discovered in this field may have applications elsewhere. For example, a strain of *B. thuringiensis* has recently been shown to produce multiple cytotoxic proteins, which are active against human cancer cells; however, it is not clear if these proteins would be safe for human use. Some endotoxins produced by Bt display antibacterial effects on some other microorganisms.

83. Some of the developments in this field could have the potential for misuse in a BW programme. For example, expertise in the transfer of *Bacillus* genes among closely related species could be utilised for malign as well as beneficial purposes, as could manufacturing expertise and facilities and field delivery systems.

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[13] For example: Strobel, G. & Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. Microbiology & Molecular reviews 67, 491-502.
Schallmey, M., Singh, A. & Ward, O. P. (2004). Developments in the use of *Bacillus* species for industrial production. *Can. J. Microbiol.* 50, 1-17.

84. *Genetically modified (GM) crops and transgenic plants.* Field releases of genetically modified organisms (GMOs) have grown enormously since the first field trial was held in 1986. From 1996 to 2005, the global area of biotech crops increased more than 47 fold, from 1.7 million hectares to 90.0 million hectares. The vast majority of releases involve GM plants. Worldwide, the principal GM crops are soybean, maize and cotton; most of these contain a single transgene that modifies the plant for herbicide tolerance or insect resistance, although plantings of twin trait cotton and maize were reported in 2004. GM plants with traits that influence virus resistance, crop quality, male sterility and disease resistance are less common, but increasing developments are expected in these areas in the future.

85. Over the past five years, there have been no marketed GMOs in the UK. There have, however, been a large number of GM plant releases approved as deliberate releases. As a result of trials one plant, a forage maize, was approved for marketing but the company has now withdrawn its application. At the present time, deliberate releases have taken a downturn, largely because of the negative attitude to GM within the UK. Many of the UK Biotech companies have moved their GM development work elsewhere.

86. Two areas of GM crop development have seen considerable research and development in the last few years, "pharma" crops and enhanced foods. Clearly, these developments will still take some time to come to commercial reality but could be at large scale field trial phase in the near future. An example of “pharma” crops is the development of an authentic insulin molecule in safflower at commercially viable levels. The company plans to continue to scale-up production for sufficient material to initiate clinical trials and file an Investigational New Drug (IND) application in the second half of 2007. In enhanced foods, GM crops containing omega-3 fatty acid are being developed as an alternative to fish as a source of this in the diet, and vitamin A enriched GM rice is being investigated.

87. A number of plant viruses (e.g. *Cow pea mosaic virus* (CPMV)*, Tobacco mosaic virus* (TMV)*, Potato virus X* (PVX)*, Tobacco rattle virus* (TRV)) are used as vectors for the expression of foreign proteins in plants. Initially these vectors were developed to examine the fundamentals of virus movement, but have subsequently been applied in several other areas of plant sciences, including the expression of vaccines and high value pharmaceuticals. In addition, modified viruses are being utilised for plant genomic studies *via* virus induced gene silencing (VIGS). As these techniques are becoming more widely used, there has been a significant shift of such projects away from plant virology to medical and other fields. Although currently used only in containment for research purposes, the increased volume of work being done in various fields increases the potential availability of these vectors for other applications.

88. Like other applications of genetic modification technologies, developments in the field of GM crops have the potential for misuse, for example by design of anti-crop agents with improved properties. The deliberate or accidental introduction of GM seeds or crops within a country that has not approved such products could have serious economic consequences due to the efforts required in detection and clean-up operations.