An Overview of the Advances Made in Biotechnology and Related BTWC Concerns

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Summary

BTWC is apprehensive of development of dual-use technologies in the areas of genetic engineering, biotechnology and microbiology, for high growth of products and processes that are capable of being used for purposes inconsistent with its objectives and provisions. These include all microbial and other biological agents or toxins, naturally or artificially created or altered, irrespective of their origin or method of production.

This paper is a collation of information available from literature in public domain.

Introduction

The Biological and Toxin Weapons Convention (BTWC) entails prohibition of the development, production and stockpiling and acquisition of biological and toxin weapons. Efforts were made to negotiate a protocol to include detailed investigation and verification to ensure that participating countries fulfilled their obligations of non-acquisition or retention of microbial or other biological agents or toxins harmful to plants, animals and humans, in types and in quantities that would have no justification for prophylactic, protective and other peaceful purposes. However, the discussions were inconclusive.

BTWC is apprehensive of development of dual-use technologies in the areas of genetic engineering, biotechnology and microbiology, for high growth of products and processes that are capable of being used for purposes inconsistent with its objectives and provisions. These include all microbial and other biological agents or toxins, naturally or artificially created or altered, irrespective of their origin or method of production. Increased knowledge of uses of many pathogenic species of micro-organisms, extraction of toxins and other biological agents and the pace of development in civil biotechnology further accentuate the possibilities of production and hostile use of biological agents. Dual-use technologies, even though they may not in principle contravene BTWC, can be used to create agents for offensive purposes. Current efforts by nations focus intensively on technologies for creating new means of protection against biological threats.

Developments in areas like physics and chemistry and engineering, computational and material sciences greatly impact progress in biotechnology. Genetic

engineering, biotechnology, toxicology, molecular biology and other related sciences have also made it possible to create a new generation of biological weapons (BW). Scientific and technological developments which would lead to transformation in organisms to be used as BW could include (i) increase in the virulence and antibiotic resistance of pathogenic agents; (ii) enhancing non-transmissible agents for airborne transmission; and (iii) creating organisms or biological products capable of acting on humans and ecosystems (parasites, insect-pests, disease vectors, etc.). Technical developments of major concern to BTWC would include:

Bio-defence: BW are capable of inflicting casualties over a large area with military effectiveness. Factors impacting their effectiveness may include use of novel agents that are not well characterized and for which there may not be vaccines or treatment. Decontamination may be difficult if deployed sensors cannot detect the agents utilized. The process of scientific and technological changes in detection, identification, diagnosis and protection provides increased capabilities to counter or protect against BW.

Genetic modifications: Advances in genetics and genetic modification techniques are making new types of vaccines feasible. There is considerable research towards genetically modified live vaccines able to immunize simultaneously against multiple antigens. Advances in knowledge of the molecular basis of antigens have led to antibody reagents of improved specificity.

Mechanism of action of micro-organisms: Understanding the mechanism of action of micro-organisms gives direct access to the mechanism of action of potential BW agents. Using molecular biology, mechanisms of virulence and infection have been identified; the same techniques may also permit deliberate manipulation of these mechanisms. Transferring certain genetic traits into naturally infectious micro-organisms can potentially create organisms of greater virulence, antibiotic resistance and environmental stability. Changing the microbes genetically could alter their immunogenicity, thereby invalidating vaccines and sero-diagnostic techniques. Otherwise harmless micro-organisms could be altered to produce toxaemia or disease: the host would continue to recognize them as innocuous and therefore not defend against them.

Micro-biological developments: Human understanding of a number of factors of direct relevance to protein synthesis and assembly has increased enormously. Remarkable progress has been made in the application of this knowledge to the production and isolation of heterologous proteins from *E. coli* and other bacteria like *yersenia*. Different expression systems are being tried.

Human Genome Project (HGP): The immediate benefits of HGP will be the identification and localization of genes causing hereditary diseases and simplification of the development of pharmaceutical drugs for treatment of hereditary diseases. It will also greatly refine their prenatal diagnosis. Investigations also provide sufficient data on ethnic genetic differences between population groups. Such data would also provide target-suitable micro-organisms to attack known receptor sites for which differences exist at cell membrane level or even to target DNA sequences inside cells by viral vectors.

Toxins and Regulators: Genetic engineering has made possible large-scale extraction and production of potent toxins, which until now were available only in minute quantities and only upon isolation from immense amounts of natural biological materials. These toxins

can now be produced in kilogram quantities in a short time with minimum cost, which could be of military significance. Rapid progress has also occurred in the modification of naturally occurring bioregulators, in understanding the physiological effect of certain essential biological substances when they are present in abnormal concentrations or under abnormal conditions, and in understanding the possible pathogenic effects of proteins.

Of special interest to BTWC are applications of biotechnology in directed molecular evolution such as genetic modification, recombinant technologies, proteomics, bio-informatics, and synthetic and systems biology. Some of these technologies vis-à-vis potential applications of consequence to BTWC are discussed in the following paragraphs.

Recombinant DNA (R-DNA) Technologies

Recent advances in biotechnology have enabled rapid and relatively inexpensive identification, characterization, mapping, manipulation and synthesis of genes and short strands of genetic material. They have helped in advancing modification of DNA or RNA in plant or animal cells or in micro-organisms like bacteria, viruses or fungi, resulting in their acquiring new unique properties. These techniques have a very wide range of applications in healthcare, agriculture and animal husbandry. They are being developed as simple to use and cost-effective; and because of their efficiency and range of potential applications, a larger number of people has access to them for use in academic institutions and industry. At the same time, these technologies have opened avenues for dual use for nefarious purposes. rDNA techniques of relevance to BTWC include:

i. **Genetic engineering:** Genetic engineering technologies have helped in manipulation of both DNA viruses and RNA viruses by inserting specific genes or nucleotide sequences into a host genome. Through reverse genetic engineering, researchers can introduce viral RNA into bacterial cells, where it can then be manipulated much more easily. Clones have already been constructed for viruses like poliovirus, yellow fever virus, H1N1 influenza A, rabies, and others. It is also now possible to reverse-engineer dangerous viruses like corona viruses, H1N1 influenza A and H5N1.

ii. **DNA synthesis:** DNA synthesis is de novo generation of genetic sequences that specifically program cells for the expression of a given protein. Technology enhancements coupled with automation and mechanization have increased the speed, ease, and accuracy with which larger sequences can be generated chemically. Examples are syntheses of genomes of poliovirus and the 1918 influenza virus. This raises concerns that more complex dangerous organisms, such as the smallpox virus, may be synthesized de novo some day.

iii. **Genome sequencing:** HGP and advances in sequencing technology have helped generate genomics data for a number of pathogenic micro-organisms. These data have helped characterize their properties and their mode of action, including virulence factors, which form the basis for new therapies, vaccines and medicinal drugs. This wealth of information can also be misused to enhance the pathogenicity of micro-organisms through genetic modification or to convert harmless organisms into a pathogenic variant

that is difficult to detect, diagnose or treat.

iv. **Fusion protein:** This technology involves insertion of a toxin in a protein, enabling it to identify and kill specific cells. This is currently being researched with a view to target and kill cancer cells. But programming the toxin differently could make it possible to kill cells essential to life.

v. **Combinatorial chemistry:** Combinatorial chemistry involves technologies to generate huge libraries of synthetic compounds with diverse properties in order to screen them for activity against biological drug targets. It contributes to drug discovery and development and to the search for better superconductors and biosensors for the detection of medically important molecules and environmental toxins. But these libraries could also be quickly and easily searched by those with malign intent for compounds with the potential to interact with endogenous physiological pathways for use as biochemical weapons.

vi. **High-Throughput (HTP) Technologies:** HTP screening used for assessment of compounds with therapeutic potential implies technologies, such as automated sequencing, mass spectrometry proteomics, transcriptomics, proteomics and metabolomics approaches coupled with advanced computational approaches to capturing information about an organism on a global scale to analyse and build a predictive model. The use of micro-organisms or higher cells in tissue culture genetically engineered to monitor a specific biological activity has made it possible to screen several thousands of compounds for the desired drug properties. This in turn has generated a wealth of genetic information on basic DNA, protein, and functional informational resources available worldwide. Some of these compounds will inevitably have activities that are toxic or affect animals and humans in harmful ways. Access to this knowledge would allow rapid identification of potentially dangerous agents.

vii. **Directed Drug Design:** Rational drug design uses structural knowledge of the drug targets to design novel chemical compounds that bind to selected sites on the surface of target molecules or mimic the structure of the target molecule, thereby competing for a receptor molecule's binding site(s). This technique is more specific than combinatorial chemistry in that it allows the scientist to target a desired site and function, and design a drug with particular properties rather than screen through a large library of compounds looking for those properties.

viii. **Synthetic Biology:** The system incorporates use of technologies in DNA synthesis, bioinformatics, and reverse genetics in order to fabricate useful microbes and learn about the underlying principles of cellular function by reducing biological systems to their simplest components and by creating models of genetic circuits. Synthetic biology allows researchers to develop a registry of biological parts and essentially create tiny programmable computers from living organisms. Re-engineered bacterial proteins when tagged with TNT are able to detect chemical or biological agent signatures or clean up environmental pollutants. The technology would also allow

creating new or existing pathogens for malicious purposes.

viii. **DNA Shuffling**: DNA shuffling involves *in vitro* homologous recombination of genes by random fragmentation and polymerase chain reaction reassembly and amplification to evolve genes and novel proteins with novel or improved functions. It allows generation of novel proteins, viruses, bacteria and other organisms in a cost-effective manner and in a fraction of the time required with classical breeding. This technology has been extended to production of small-molecule pharmaceuticals, pharmaceutical proteins, gene therapy vehicles and transgenes, bacterial and viral vaccines and laboratory animals. On the obverse side, the technology is open for adoption to generate potentially lethal viruses and bacteria.

ix. **Artificial synthesis of viruses:** Small viruses are being synthesized, based on availability of genetic code. Thus, a complete, fully functioning polio virus was synthesized in 2002 by a group of American researchers. Constructing viruses that are difficult to obtain remains a possibility as long as the genetic code is known. For larger viruses, technologies like Polymerase Assembly Multiplexing using microchip arrays are being standardized. For still larger viruses like smallpox, technologies are being tried to introduce DNA changes into the genome of other family members of poxviruses, which may lead to a change in the host range of these viruses to include humans. These developments may be disturbing: the structure of viruses is extremely simple compared to bacteria, and immunity – either natural or conferred through vaccination – has over the years disappeared in the human population. New technologies may also be able to build more complex viruses is due course of time.

x. **RNA interference (RNA*i*):** RNA*i* technology has the potential to suppress cellular production of certain proteins in the physiological processes to halt undesirable processes or stimulate desirable ones of importance in therapeutic use. Efforts are being made to develop this tool to target disease-causing genes and proteins that are inaccessible to conventional drugs. Short, single-stranded nucleic acids (aptamers) and protein-DNA chimeras (tadpoles) have high binding affinity and are being explored in animal models for their potential to be used in the inhibition of blood clot formation and treatment of age-related ocular degenerative changes. The effectiveness of small interfering RNA (*si*RNA) as antiviral has been demonstrated *in vivo* for Ebola virus infection of primates. RNA*i* technology also has major implications in the functioning of the mammalian cells. Studies have demonstrated the role of siRNA in animals' regulation of gene expression by micro-RNA molecules (*mi*RNA), which interact with cellular RNAs to suppress certain proteins and silence the expression of target genes. siRNA are otherwise also sufficiently active to function as potent antiviral agents to modulate gene expression in adult animals.

In plants, RNA*i* is commonly being used as antiviral defence mechanism. *si*RNA molecules, initially identified in plants, are able to promote the sequence-specific degradation of messenger RNAs and are therefore able to suppress gene expression.

Theoretically, the approach of using RNA*i* technology has wide implications for developing therapeutics for a number of diseases and as a research tool in functional genomics. The technology also allows long-term, efficient silencing of an allele that segregates with ethnicity. Silencing of genes that function in innate immunity could lead to conditions that mimic, at least superficially, natural disease. The whole approach of RNA*i* defines a significant change in the utility of genetic weapons technology. RNA*i* would have dual-use implications. New therapeutic options for treating cancer and other diseases could also lead to manipulating gene expression to do harm.

Human Genome Project (HGP)

HGP, completed in 2003, discovered all the estimated 20,000-25,000 human genes and makes them accessible for further biological study. It also determined the complete sequence of the three billion DNA subunits bases in the human genome. The technology and resources generated by the project and related genomics research have already created a major impact on research across the life sciences. The potential for commercial development of genomics research presents a wealth of opportunities, which are now being exploited.

Information generated by HGP has wide applications in biomedical research leading to improved diagnosis of diseases, drug design, pharmacogenomics, gene therapy, etc. Microbial genomics research has led to sequence of bacteria useful in energy production, environmental remediation, toxic waste reduction, and industrial processing. Other applications are in the areas of environment risk assessment for individuals from toxic materials, DNA forensic sciences including fingerprinting, pollutants, breeds, etc. Genomic studies are also directed towards study of evolution, migration and mutation with age. Understanding plant and animal genomes will help in studies of disease patterns in plants and animals, disease-resistant plants and animals, reduced management costs, and better, nutritious and pesticide-free foods.

However, HGP has also raised serious concerns. Elucidation of the structure of human DNA and the functioning and regulation of genes makes it possible to identify markers which determine the racial and ethnic differences between people and target the genetic makeup and ethnicity of specific groups of people. A study in the US on the Y-chromosome and mitochondrial DNA in populations from different regions has suggested that the data generated could be used for developing methods to selectively disturb cellular respiration and energy exchange, sexual reproduction and a number of other important functions connected with the Y-chromosome.

Data analysis of the human genome showed that hundreds and even thousands of sequences obtained could serve as targets for selective BW based on the difference in frequency of genetic markers between races and difference in the frequency of polymorphisms. A recent study in Taiwan has discovered that Severe Acute Respiratory Syndrome (SARS) can be associated with specific genetic profiles.

Genomics and Proteomics

Genomics

After completion of the human genome and rat sequences in HGP, there have been dramatic advances in the sequencing of mammalian genomes. Several hundred eukaryotic genome sequencing projects are underway. Technology developments,

including capillary sequencing machines and high-density overlapping oligonucleotide chips, allow rapid re-sequencing of genomes. Genome sequences are now available for hundreds of pathogens, especially bacterial and viral pathogens of public health importance. The technology is being used to study the extent of sequence variation that exists within a species. Genome sequencing of pathogens using HTP screening has helped in better understanding of antibiotic resistance and emerging and re-emerging bacterial and viral diseases.

The International HapMap Project, initiated in 2002, is a multi-country effort to identify and catalogue genetic similarities and differences in human beings and a major effort toward identifying genes affecting health, disease, and individual responses to drugs and environmental factors. Information generated under the project would be available in the public domain and could be exploited to target specific groups with harmful agents. Identifying populations most vulnerable to certain diseases also makes them vulnerable to isolation as potential targets.

Use of advanced sequencing technologies has also led to mapping of the molecular signatures of the bioregulatory systems of the body and how these regulatory pathways respond to disease-induced disturbances. The information, which is critical as target for therapeutic and preventive intervention or manipulation, can also be used for a novel biological attack. Significant advances have also been made in genomic medicine, where patient-tailored treatment of diseases is prescribed based on analysis of his/her genetic makeup.

Proteomics

The proteome is traditionally studied through a combination of gel electrophoresis and mass spectrometry. Use of HTP automated technologies and fractionation strategies coupled with mass spectrometry and gel separations has made it possible to detect low levels of proteins or sub-groups of proteins and analyse protein mixtures. Proteomics can differentiate isolates or strains and greatly enhances our knowledge of host-pathogen interactions, protein-protein interactions, host response to infection and pathogenesis. Proteomics also has applications in identification of candidates for diagnosis, therapy, detection systems and vaccines; this knowledge could be exploited for non-peaceful purposes.

In comparative proteomics, proteins from different growth conditions, strains or species can be labelled and differentiated using mass spectrometry to identify proteins having 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.

The 'immunome': The study of immuno-dominant proteins which trigger immune response to infection and that of 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 enables the rapid analysis of hundreds of proteins in parallel. These techniques are being used to detect and diagnose biomarkers as well as possible vaccine candidates.

Synthetic biology: In most cases, with the exception of RNA viruses, DNA acts as a blueprint producing all the essential elements required for a functional biological

system. Technological gains in chemical DNA synthesis have facilitated synthesis of DNA constructs, which has made de novo synthesis of some small viruses a reality. Efforts are being made to synthesize large viruses' constructs and chemical synthesis of bacterial genomes.

Protein expression and production technologies: Progress in rDNA technology has allowed cloning directly into expression vectors and high-fidelity easy transfer of genes between expression vectors. This has decreased the time and efforts required to clone a target gene and reduce the level of expertise required. Advances in vectors and plasmids to enhance the expression of soluble and problematic proteins have raised the potential to generate significant quantities of a protein to be produced from synthetic DNA. Expression systems are commercially available for production of proteins within bacteria, yeast, plants, filamentous fungi, insect and mammalian cells.

Synthetic biology has allowed construction of functional genetic circuits in cells and micro-organisms, merging engineering approaches with biology. Sets of standardized genetic building blocks are developed and suitably assembled to perform specific functions such as detection of toxic chemicals, explosives and biological agents, disease diagnosis and therapeutic intervention, production of pharmaceuticals, bioremediation of pollutants, energy generation, etc.

Chemical DNA synthesis facilitates efficient and cost-effective production of natural products with potential uses in therapeutics or for other beneficial purposes. The technology is of particular benefit especially when analysing proteins from dangerous organisms, in that the gene can be chemically synthesized, and 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. But this technology has the potential for misuse, since exotic pathogens are exploited without having the necessary infrastructure.

The rapidly developing field of synthetic biology also has the potential to create risks for society, due to either unintentional consequences for health or the environment or deliberate misuse.

Advances in recombinant technology have led to increasing focus on the production of proteins from pathogenic hosts in micro-organisms which are more easily and safely handled on a large scale. High containment facilities, cost and skilled labour make it a distinct possibility in reality. An example is a recombinant protective antigen as the basis of a new-generation anthrax vaccine.

Gene Therapy

Gene therapy uses healthy genes to treat or prevent disease by inserting a normal gene into the genome to replace an abnormal, disease-causing gene. It typically uses a carrier molecule or vector, such as a harmless virus, to deliver the healthy gene to the target cell. This technology is also used to introduce new genetic material into the cells of individuals with the specific target receptors or to inhibit the expression of endogenous genes. The technique, though in an experimental state and yet being standardized, has major potential benefits for human and animal health in the treatment of genetic diseases and in the modulation of gene expression such as suppression of inappropriate immune reactions. The silencing of alleles associated with ethnicity now appears theoretically possible.

Studies however have also reported setbacks in the development of vectors for delivery of such therapy. Repeated application of high doses of adenovirus vectors is apprehended to lead to immuno-pathology; lentivirus/ retrovirus vectors may also be associated with cancer-causing effects. Safety considerations also have the potential for misuse, providing the opportunity to achieve long-term expression of a deleterious gene in a target population.

Development of affinity media for both laboratory- and manufacturing-scale purification of affinity-tagged protein results in high yields of a specific product. New systems are now available for laboratory-scale automated purification of several proteins simultaneously.

Continuing progress in processing equipment for the pharmaceutical and biotechnology industries has led to the emergence of portable bioreactors featuring disposable contact materials that eliminate cleaning, sterilization and validation and can easily be used for virus production, monoclonal antibody production, cell culture and production of human therapeutics. But these developments are potentially open for misuse. Successful protein expression and production strategies, including portable systems, can facilitate dual use: benefits to the pharmaceutical and biotechnology industries as well as potential BW production. Transgenic plants could 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. Being natural bioreactors, they eliminate the need for much sophisticated equipment for producing them in large amounts.

Systems Biology

Systems biology or integrative biology involves the application of systems – and signal-oriented approaches – to understanding inter- and intracellular dynamic processes. Tools that could be used to manipulate complex biological systems include gene silencing, novel binding reagents like nucleic acid, peptide aptamers, engineered antibodies, immune modulators, etc. Advanced HTP tools are used to study the complex interactions involving networks of molecules, including DNA, RNA and proteins to analyse cellular regulatory networks and pathways and genomic and proteomic setup. These tools have a great impact in improving the predictive accuracy of models of biological systems, which allows physicians better management of preventive and therapeutic measures based on genotypic and phenotypic makeup of individuals. The same advances could also make it easier to identify ways to maliciously manipulate biological systems.

Micro-fluidics and micro-fabrication technologies have been used in manipulation of a wide variety of processes at miniaturized scales using automation. These technologies find application in DNA analysis, immunoassays, cell analysis and measurements of enzyme activity and have greatly enhanced the diagnostic ability of disease outbreaks. Such technologies are applicable in both naturally occurring and deliberately created conditions and open the road to identify ways to maliciously manipulate biological systems, thus introducing novel aspects of future bio-defence and bio-threat agents.

Host-pathogen Relationship

Microbial genome sequencing programmes have led to better understanding of the pathogenicity and virulence factors contained in the genetic makeup of micro-organisms. They have greatly improved the knowledge of how several micro-organisms effect pathogenesis through characterization of their discovered virulence factors. There is increasing awareness that many of these virulence factors act in concert with one another and with host factors.

There has also been an increase in the studies of the response of the host to infection in order to fully characterize the virulence of micro-organisms. Although DNA micro-arrays 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. 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 weakening the immune response to specific pathogens.

Vaccines and Therapies

Market requirement of vaccines and biological agents has grown tremendously. In India, the requirement of therapeutics has registered an annual increase of between 15 and 20 per cent. Global research continues towards improvement and development of new vaccines and technologies. New micro-organisms, new molecules, carrier systems and delivery modes are being adopted using new principals to improve the efficacy of biological materials. Vaccines and diagnostics are available for almost all the known disease conditions. There is an equal interest among nations to develop or acquire vaccines and biological agents for defence purposes since these show possibilities of discovery of compounds with potential for misuse.

Significant advances in technologies, accompanied by sophistication in engineering processes, have helped in the diagnosis of and development of antiviral drugs, especially reverse transcriptase inhibitors, DNA polymerase inhibitors or protease inhibitors for treatment of a range of infectious and non-infectious disease like HIV, hepatitis B and C and malaria, influenza viruses H1N5, H1N1, etc. Vaccines and anti-viral drugs are also being developed of defence interest, including anthrax, poxvirus infections, etc. As we have seen earlier, recombinant and gene technologies have also been used to develop DNA vaccines and sub-unit vaccines and artificially produce small viruses and other oligo-nucleotides.

The use of phage/ribosomal display technologies, combinatorial chemistry, molecular modelling and HTP screens has helped in the discovery and design of potential therapeutic peptides. Specific peptides for a particular cell surface receptor bring about an intracellular effect on a target cell and/or a physiological change in the target organism. Some of these peptides are increasingly in use to target specific markers in oncologic conditions. Immuno-modulators such as cytokines and non-specific immune stimulators strengthen the immune defence system to protect against a range of pathogens. Commercial-scale synthesis of peptides can also provide significant quantities of desired pathogens produced in

recombinant micro-organisms or in transgenic plants or animals.

New antiviral drugs have been developed using reverse transcriptase inhibitors like VIREAD (tenofovir disoproxil fumarate) for treatment of HIV, and DNA polymerase inhibitors like HEPSERA (adefovir dipivoxil) to arrest replication of the virus responsible for chronic hepatitis B.

Delivery of sufficient quantities to the appropriate target cells or tissues across the blood/brain barrier is a significant challenge to the development of therapeutic peptides. Vaccine delivery systems using new aerosol generating devices and improved adjuvant, live vectors and micro-encapsulation technologies are being developed commercially for effective delivery of therapeutics. Therapeutic use of targeted monoclonal or polyclonal antibodies has proved useful to prevent and treat virus-induced diseases.

Penetration enhancers with ability to penetrate the skin or mucous membranes to improve the absorption of medicinal drugs also lower the threshold at which micro-organisms or toxins become harmful. The potential benefits of advances in technology for delivery of drugs and vaccines also raise the potential for misuse, such as making new routes for the delivery of BW, especially immune modulators and immune stimulators to attack the immune system. Such methods could also result in the intentional or unexpected discovery of compounds with potential for misuse.

Bio-regulators

Bio-regulators or Physiologically Active Materials (PMNs) are naturally occurring substances present in very low concentrations in the body. They conduct biological activity that regulates and coordinates a number of physiological processes, including cardiovascular function, respiratory system, nervous system and immunity. Bio-regulators include hormones, signal molecules, enzymes and inflammatory mediators. Knowledge in this field has made it possible therapeutically to intervene in order to boost desirable processes or slow down harmful ones. Advances in the areas of life science research on bio-regulation of physiological functions has led to characterization of bio-regulators and the search for promising compounds for pharmaceuticals. There has been a tremendous increase in the identification of peptides, both toxins and bio-regulators that control biological processes. An excess of certain regulators is found to lead to sleep disturbance and behavioural changes. Administering other regulators will lead to autoimmune reactions or, because they can affect blood pressure or cellular ion homeostasis, to heart rhythm disturbances, organ failure, paralysis, coma and death.

PMNs are aimed specifically at the cells to ensure the first line of antiviral and anti-tumorogenic protection. Studies have also been directed at explaining interactions in different receptor systems and determining new targets connected with the disturbance of physiological concentrations of endogenous PMNs.

The possibility to manipulate toxins or bio-regulators or to produce them in pure form in large quantities opens up new perspectives that have to be considered with implications for BTWC. Bio-regulators are considered to pose a serious threat of being used for illicit purposes due to the increased understanding of inter- and intra-cellular processes and control of central biological processes of mammalian systems, including human.

Production Biotechnology

Industrial application of biotechnology for large-scale production of micro-organisms and cell products has increased tremendously. The new techniques simplify the large-scale production of all kinds of bio-engineered products and organisms in more modest sized plants at rapid speed and cost-effectiveness. This has been beneficial from the point of view of public health, food and agriculture, but it has also increased the potential of misuse for developing and large-scale production of potential agents to be used as BW.

Genetic modification of micro-organisms or plants has helped overproduction of proteins and made the fusion of proteins economical, efficient and quicker. Heterologous expression systems and associated production technologies using yeasts, bacteria and fungi are engineered to produce recombinant proteins and therapeutic compounds in bulk scale for legitimate commercial purposes. Optimization of these technologies is equally applicable in large-scale expression and production of proteins and toxins suitable to be used as BW, such as botulinum. Technologies have been optimized for production of live attenuated vaccine for several bacterial and viral pathogens having key virulence factors removed, either through serial passage or through targeted genetic manipulation. The production technology required to produce a live attenuated anthrax vaccine is identical to that required to produce live virulent anthrax agent in bulk.

Toxin Production

Worldwide consumption of toxins for medical therapy and scientific research has reached a level of hundreds of grams and kilograms per year, and the projected future growth of toxin therapies will require tens to hundreds of kilograms of material annually. It is extremely difficult to distinguish between production of medical or militarily useful quantities.

The major impact of genetic engineering relevant to BTWC is the possibility of large-scale production of toxins. Improvements in biotechnology have led to the production of potent toxins, which until now were available only in minute quantities and only upon isolation from immense amounts of natural biological materials. These toxins can now be produced in kilogram quantities within a short time with minimum cost, which could be of military significance.

Use of rDNA techniques permits the transfer of genetic material between widely divergent species. The increase in knowledge of many of the pathogenic species of micro-organisms, and knowledge of toxins and other biological agents and the continuing pace of developments in civil biotechnology areas, has increased the possibilities for production and hostile use of biological agents affecting the normal environment around the human being.

Improvements in technologies have helped production in significant quantities of recombinant animal toxins that are difficult to isolate from a natural source. The technologies as well as the quantities produced have obvious implications as BW agents. Also, information gained in studies on the utility of toxin sub-units in targeting therapeutic agents to specific cells has the potential to be exploited for targeting harmful agents.

Much interest these days has been generated in identification and purification of toxins from marine resources having therapeutic potential. Though isolated in small quantities, they have already been shown to

have potential of exploitation for generating significant amounts of bioactive substances of both therapeutic and harmful effects. Recently a bioactive peptide, a synthetic conotoxin compound produced by cone snails, has been licensed for use in the treatment of severe chronic pain.

Botulinum toxin is a therapeutic for a number of disease conditions. The catalytically active and toxic A-subunit portion of these toxins conjugated with antibodies raised against specific antigens found on the surface of tumour cells is used for site-directed anti-cancer therapy. B-subunit toxins are being exploited to study intracellular delivery mechanisms like delivery of therapeutic agents to neural cells for the treatment of neural dysfunctions. It is also well known that botulinum toxin is a potential bio-agent for military use.

The potential benefits of advances in technology and delivery of drugs and vaccines also raise the possibilities of misuse, to improve delivery systems for BW agents, especially immuno-modulators and immuno-stimulators for attack of the immune system.

Computational Biology and Bioinformatics

Bioinformatics is the application of large-scale data analysis techniques to the life sciences, encompassing such areas as biology and medicine, computer science, statistics, mathematics and physics. Bioinformatics has become an essential component of modern biology in academic, government and industry research sectors since a lot of biological data are being generated using techniques of genome sequencing, proteomics and HTP data collection. To interpret and utilize these data, bioinformatics has continued to develop in parallel, supported by advances in computer technology and the accessibility of data and tools via the internet and on computers. Bioinformatics is working with HTP technologies to make it easier to create novel structures and substances from biomaterials and is creating new scientific and commercial opportunities.

Complete genomes of an increasing number of organisms have been sequenced and characterized. Genebank public repository containing records of organisms' genetic content doubles in size every month. Together with computational biology, bioinformatics has made it possible to predict properties and complete metabolic pathways from sequence information generated from the genetic material isolated from different environments. Bioinformatics is also being used in the analysis and modelling of pathogens, understanding of their pathogenicity and virulence, and in the study of host-pathogen relationship vis-à-vis antibiotics. Bioinformatics is also being used successfully to produce predictions for vaccine candidates, virulence factors, drug targets and novel therapeutics. Using the genetic information, compounds have been prepared in the laboratory which mimic the natural organisms. The technical developments in chemistry have made it possible to isolate and characterize compounds from very complex environments even when present in very low amounts. These advances could also be misused in the development of pathogen strains with increased virulence or drug resistance, or with improved stability to assist survival within the environment. The complementarities and synergy of technologies used in biotechnology, nanotechnology and information technology are converging in ways that will enable life processes to be manipulated, with far-

reaching implications and great potential for nefarious and disastrous outcomes.

Nanotechnology

Nanotechnologies are defined to include designing, characterization, production and application of structures, devices and systems by controlling the shape and size of materials at nanometre scale. Advances in the ability to produce and characterize materials at nano-scale have resulted in materials with novel and useful properties having great potential to bring benefits to many areas of research and application. This technology combines biotechnology, synthetic biology and information technology to design molecular structures capable of performing a wide range of functions.

Application of nanotechnologies in materials and technologies, electronics and micro-systems and clean technologies has already benefited day-to-day requirements. In the life sciences and medicines, nanotechnology has wide applications in bio-nano materials, bio-sensors, biomarkers and nano-particles, cancer diagnostics, imaging and treatment, drug delivery and therapeutics, including gene therapy and nano-medicine. A prime example of the use of nano-particle science is for creation of novel and highly efficient delivery systems for difficult-to-deliver biologically active compounds.

Nano-biotechnology has particular promise in disease diagnosis, including nano-particles capable of targeting specific diseased cells, containing both therapeutic agents and a sensor that regulates their release into the cell. Nanotechnology thus has direct application to drug discovery to study drug-receptor interactions at the single molecule level. Other applications include 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. Micro-sensors or implants made of biological material are smaller and more effective than current implants. Current applications also focus on using custom nano-scale materials for *in vivo* applications such as molecular imaging and detection, reporters for therapy efficacy determination, multifunctional therapeutics, disease prevention and control. As a growth industry nanotechnology commercially is expected to eventually put the pharmaceutical market in the shade. Production, delivery, and packaging technologies that allow biological systems to be manipulated in a defined, deliberate manner are evolving very quickly to serve the pharmaceutical, agricultural and healthcare fields. Some of these technologies have not been traditionally viewed as having relevance to future biological threats.

The rapid expansion of nanotechnology, however, has also raised new challenges in the safety, regulatory and ethical domains that will require wider consideration. Interactions between nano-particles and living cells and material provide for the synthesis of novel substances, which possess greater toxicity and irreversibility of action than any identified previously. This ability of nano-particles to easily pass through human biological barriers when combined with qualitatively new toxicological properties, and its irreversible consequences, could lead to the creation of a new class of physiologically active materials that could become the basis for developing a new type of lethal BW. For emerging technologies in this field with potential for development of novel or enhanced biological agents with improved delivery methods, it is difficult to predict the outcome of many research areas and thus the impact on potential BW applications.

That nanotechnology has the potential to deliver toxic agents maliciously is evidenced by the fact that the European Commission

has published a Nanotechnology Action Plan and the OECD (Organization for Economic Cooperation and Development) is working to promote international cooperation in the health and environmental safety-related aspects of manufactured nano-materials. Various national, regional and international stakeholders are also interacting with each other in the same direction.

Polymers

Polymers are substances with a high molecular mass composed of a large number of repeating units (monomers). Biological macromolecules or natural polymers include carbohydrates, starch, cellulose and glycogen and chitin. Advances in technology have helped in commercial development of synthetic polymers from petroleum products like polyethylene and nylon. Synthetic polymers made out of glycolic and lactic acids and other biodegradable materials have shown properties of stimulus responsiveness to the environment and can be used for a variety of purposes related to biotechnology and biomedicine. Known as smart polymers, they can detect even slight changes in their environment. Smart polymers have been in increasing use in the areas of diagnostics, biosensors, pollutant detection, food contaminants, etc. Smart polymers are known to be among the best drug delivery systems, as smart polymer matrices release drugs by a chemical or physiological structure-altering reaction, often a hydrolysis reaction resulting in cleavage of bonds and release of drug as the matrix breaks down into biodegradable components.

Some recent smart polymer applications include use of molecular imprinted polymers in combination with peptides in diagnosis of mycotoxins, use of nucleic acid biosensors using polymer transducers for rapid detection and identification of biological pathogens, and use of micro-fluidic chips in combination with monolithic porous polymers for extracting biological and chemical toxins from air, soil and water samples.

Drug Delivery

A number of new matrices have been identified as carriers for prolonged and sustained delivery of drugs and pharmaceuticals. At the same time, routes of administration of drugs and vaccines ensure maliciously target delivery to infested areas. Advances made in areas like drug design, synthetic biology, systems biology, aerosol technology, nanotechnology, micro-encapsulation, etc. have advanced the knowledge for targeted delivery of drugs but have also provided insights to systems required for nefarious use: these need to be assessed.

Nano-emulsion technologies: Significant progress has been made recently in the use of large porous particles (LPP) for delivery of drugs through adsorption in the lungs. LPP are considerably larger than the size regarded as optimal for inhalation and deep deposition. But due to their low density, LPPs are inhaled and the drugs are delivered efficiently. The LPPs' large surface area, in the size range of 25 to several hundred nano-metres, can be used to carry a large number of small particles. The concept is frequently used to coat LPPs with nano-particles carrying drugs for optimized aerosol delivery. On the obverse side, these techniques could potentially be used to develop highly efficient aerosol delivery systems for micro-organisms (viruses and possibly also small bacteria), toxins or chemical compounds for BW purposes.

Micro-encapsulation

Micro-encapsulation entails prolonging the shelf life of micro-organisms or proteins in the body or the environment by coating or enclosing them in a biopolymer capsule. Minuscule solid particles, liquid droplets or gas bubbles are enveloped in protective coating comprising of any of a number of compounds like organic polymer, hydrocolloid, sugar, wax, fat, metal or inorganic oxide. The coating protects against their evaporation, oxidation and contamination, thus ensuring that they are released over a longer period of time. The technique has application in controlled and delayed drug delivery, including micro-encapsulated proteins and peptides and engineered and live cells for therapeutic purposes. Considerable knowledge to achieve these aims efficiently has been generated. Micro-encapsulation of drugs has been exploited to carry micro-organisms to selectively target viruses or bacteria as vectors for delivery of genes and proteins and as viral delivery vectors to insert genes into chromosomal DNA. At the same time, the knowledge generated helps in the harmful spread of micro-encapsulated peptides, proteins including toxins and bioregulators, and micro-organisms while avoiding environmental exposure to ultraviolet light and other oxidative stresses.

Aerosol Technology

Pharmaceutical and biopharmaceutical industry is constantly looking for new technologies for administration of therapeutic compounds to treat patients using different vehicular routes. Aerosol technology is among the widely accepted delivery vehicles to deliver biologically active organisms or compounds, including therapeutic molecules, to target structures. New advances in the field of aerosolization include micro-encapsulation. Different delivery vehicles for micro-encapsulation and sustained release of particles have also been used, based on the particle size of the aerosolized substance and its ability to be delivered directly into the bloodstream. Aerosolization provides efficient and regulated delivery of therapeutics directly to the target. Potential delivery platforms also include the use of bacterial plasmids or viral vectors for cloning the genes encoding bio-regulators, transgenic insects for production and inoculation, nano-scale delivery systems, and liposome or biodegradable micro-spheres for controlled release. Examples are propellant metered-dose inhalers, dry-powder inhalers, and nebulizers that are frequently used to deliver drugs directly to the lungs and circulatory system in asthma patients. Drug particles used in these technologies provide the enormous adsorptive surface of the lungs to enhance their effectiveness. Aerosol-based ano-emulsion technology is also being used to deliver insulin directly through nasal absorption. Nevertheless, all these technologies also raise concerns about the delivery of bio-regulators and other toxic substances through use of aerosol technology.

Diagnostic Technologies

Advances in diagnostic technologies involve equipment and materials that help detect with more precision, accuracy and speed minute quantities of pathogens or genetic makeup having the highest specificity and sensitivity for any disease conditions, including viral and bacterial infections. Rapid and specific detection of minute quantities of DNA has been achieved using reverse transcriptase polymerase chain reaction (RT-PCR), a variant of polymerase chain

reaction used in generation of many copies of a DNA sequence. A number of gene probe systems for array have been developed along with strategies to overcome problems of non-specific hybridization, including a wide range of fluorescent detection molecules. Easy-to-use hand-held devices have been developed commercially for rapid diagnosis and environmental sampling.

Antibody-based technologies have been developed and are commercially available for diagnosis of almost all disease conditions, including viral, bacterial and parasitic infections. Diagnostics have been developed using antibodies/antigens labelled with enzymes, isotopes and fluorescence to achieve detection by colour assays, fluorescence or luminescence. Antibody-based biosensors are also being used for disease detection and environmental monitoring.

Advances in antibody-based production technologies use matured hybridoma cell lines. Monoclonal antibodies – murine-derived, chimeric and humanized – have been developed. Advances in phage display technologies allow production of single-chain antibody systems that are more robust and capable of operating in harsher environments and over greater temperature ranges than conventional antibodies. Other technologies, including oligosaccharide and array-based chips for carbohydrate detection, nano-particles including gold nano-particles being used as matrix in detection technologies for biological agents and the presence of toxins, optical biosensors for real-time detection and identification of airborne antigen, bioluminescence techniques for generic detection and Dip-stick technologies based on lateral flow devices offer different methods of detection and identification. New technologies of micro-fluidics and micro-fabrication entail a wide variety of processes and manipulations carried out at miniaturized scales, usually through automation. Micro-fluidic or "lab-on-a-chip" technology is potentially useful in point-of-care diagnostics, including DNA analysis, immunoassays, cell analysis and enzyme activity measurements. Sophisticated, miniaturized diagnostic systems have immense ability to identify and respond to disease outbreaks, whether naturally occurring or deliberately caused.

Animal Healthcare

Initiatives have been taken worldwide, through multi-pronged approaches, for improvement of animal health through programmes in the areas of diagnosis of exotic diseases like West Nile Fever (WN), salmonellosis, PPR, blue-tongue virus, bovine tuberculosis, etc. and vaccine development for foot-and-mouth disease (FMD), infectious bovine rhinotracheites (IBR), rabies, anthrax, new castle disease, etc. Major initiatives have also been taken in areas of genomic analysis, molecular characterization including sequencing, genetic mapping, expressed sequence tags, comparative sequence analysis, etc.

Outbreaks of infectious diseases in animals have regularly been connected with terrorist activities or offensive BW programmes. Examples include: bird influenza in 2004 and 2005, the 2003 epidemic of atypical pneumonia, and the 2001 outbreak of FMD. Recent epidemics of SARS, Avian Influenza, Swine flu, and FMD outbreak in the UK have necessitated countries to develop their own veterinary surveillance strategy and policies and operational guidelines for control and management of outbreaks of exotic animal diseases, including standardization of laboratory technologies for detection of zoonotic diseases of public health importance. The veterinary surveillance

strategy also records patterns of emerging diseases and alterations in endemic diseases.

The knowledge gained in development of vaccines and other intervention strategies for safeguard of animal health and the magnitude and effects of global animal and zoonotic disease outbreaks has raised public awareness further, as also the potential for use of such agents for hostile purposes. Genetic alteration or modified vaccines would make such outbreaks more difficult to manage. Safeguard of animal and public health and surveillance and management of exotic diseases would need to be based on differentiation of natural, accidental or deliberate release of biological agents into the environment.

Plant Pests and Diseases

Agri-biotechnology has been growing steadily despite controversies related to "transgenics". The spectrum of biotechnology applications in agriculture includes generation of improved crops; microbes; use of molecular markers to tag genes of interest; accelerating of breeding through marker-assisted selection; fingerprinting of cultivars; DNA-based diagnostics for pests/pathogens of crops; and assessment and monitoring of biodiversity. The majority of commercially available genetically modified (GM) crops have agronomic advantages like herbicide tolerance or insect resistance. Strategic research areas include expression profile; functional validation; signal transduction; transgenic; and genetic enhancement. But the use of modern technologies to improve the quality and quantity of farm products as well as food products raises possibilities also for modifications leading to agro-terrorist activities. Farms and food supply remain among the most exposed targets, and impossible to guard adequately.

Climate changes and human population growth are expected to induce increased pest and disease problems, particularly due to invasive organisms. The best efforts at plant protection are also not able to constrain development of alien pests and diseases. Research in biological pest control has resulted in increased interest in the development of more refined dispersal models for biological aerosols. At the same time, the knowledge gained on persistence and ecological effects when releasing genetically modified organisms would also be of value while considering the effects of releasing BW agents in the environment. By using genetic engineering it may also be possible to programme the survival of a released bacterial population. Microbial pathogens could also be genetically engineered to maximize infectivity and pathogenicity. Likewise, they could be modified to increase or decrease their environmental stability and persistency, thereby cancelling out vaccines and sero-diagnostic techniques.

Research in biological pest control has resulted in increased interest in the development of more refined dispersal models for biological aerosols. The knowledge gained on persistence and ecological effects when releasing genetically modified organisms would also be of value while considering the effects of releasing BW agents in the environment. With genetic engineering it may also be possible to programme the survival of a released bacterial population. Knowledge gained in the area of bio-pesticides could in principle be misused by an aggressor intending to attack crops. Some aspects of the dissemination technology would also be relevant to the deliberate release of organisms or toxins harmful to humans or animals.

Genetic Modification in Plants

Field releases of GM crops and transgenic plants have grown enormously since the first field trial was held in 1986. The principal GM crops being used are soybean, maize and cotton. These crops mostly contain a single transgene that modifies the plant for herbicide tolerance or insect resistance. A number of other GM plants are being tested for traits that influence virus resistance, crop quality, male sterility and disease resistance.

There is considerable interest and a lot of research has been going on in the development of crops with enhanced foods and pharma crops. In enhanced foods, GM crops containing omega-3 fatty acid are being developed as an alternative to fish source in the diet, and vitamin A enriched GM rice (golden rice) is being investigated in field trials. In pharma crops, GM crops containing pharma-active molecule, termed as edible vaccines, are being investigated as bioreactors for producing sufficient materials to initiate clinical trials. An example is development of an authentic insulin molecule in safflower.

Advances in expression technologies have led to use of some of the plant viruses like cowpea mosaic virus, tobacco mosaic virus, potato virus X, and tobacco rattle virus as vectors for the expression of foreign proteins in plants. These vectors have been applied in several areas of plant sciences, including the expression of vaccines and high-value pharmaceuticals. Modified viruses are being utilized for plant genomic studies via virus-induced gene silencing, leading to their use in medical and other fields. But the potential availability of these vectors for applications as bioreactors for developing useful bio-products for human beings may also lead to their potential use to develop toxic compounds.

Like other applications of GM technologies, developments in the field of GM crops have the potential for misuse. For example, anti-crop agents can be designed 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. Also, microbial pathogens could be genetically engineered to maximize infectivity and pathogenicity. Likewise, they could be modified to increase or decrease their environmental stability and persistency. These developments have potential to be applied for beneficial peaceful purposes, but also may be applied maliciously.

Bio-pharming

Plants and animals are used to produce bioactive molecules intended for industrial products and pharmaceuticals. Bio-pharming enables production of vaccines and antibodies that otherwise are too expensive or inefficient to produce using conventional production methods. The same technologies, however, are helping the scientists to explore plants as a cost-effective way to produce agents capable of bio-warfare or as antibodies for use against potential bio-warfare agents. Genetic modification of plants renders them more lethal than non-transgenic crops. Large quantities of bio-regulatory or other toxic proteins having potential to be used as biological agents can be produced in a short time, eliminating the risk of discovery.

Biological Pest Control

Significant progress has been made in the study of microbial agents for the purpose of biological control of pests and diseases of plants. Advances in molecular biology

research have the potential to revolutionize the efficacy of bio-control agents. This has caused a paradigm shift in the use of chemical crop protection technologies. Efforts are also being made to explore vast sources of largely untapped naturally occurring organisms with the potential to provide new toxins suitable for pest control and their formulation. The availability of novel synthetic chemicals with a more benign environmental and health profile remains a factor for consideration.

Most important research and development has focused on *Bacillus thuringiensis (Bt),* towards the worldwide growth of transgenic Bt crops. Further efforts are aimed at finding new genes and toxins from Bt strains with more effective pesticide toxins, to increase the range of targets that can be controlled using either conventional bio-pesticides or transgenic methods. In this effort complete genome has been sequenced of the entomo-pathogenic bacterium, *Photorhabdus luminescens,* as a source of new genes for insect control. 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 bio-pesticides. A novel strain of a new species of bacteria, *Bacillus nematocida*, has been discovered with activity against nematodes. Some endotoxins produced by Bt display antibacterial effects on some other micro-organisms.

Molecular technologies have also been utilized to enhance the insecticidal toxicity of Bt toxins by combining the attributes of the Bt toxin with other micro-organisms, including baculovirus-based systems and other bacteria. Delivery of toxins via other microbial expression systems is a possible alternative to the production of transgenic plants. But these developments also have the potential for misuse in a BW programme: expertise in the transfer of Bacillus genes among closely related species could be utilized for malign purposes, as could manufacturing expertise and facilities and field delivery systems.

Research in biological pest control has also increased the hunt for development of refined aerosol models for effective dispersal of biological agents. Using genetic engineering technologies also makes it possible to programme the survival of a released bacterial population and the effect in the environment. The knowledge of persistence and ecological effects would also be of value while releasing the genetically modified organisms. At the same time, knowledge gained in the areas of dissemination technology of bio-pesticides could be misused by an aggressor to release organisms or toxin harmful to crops, animals and to some extent in humans.

Bio-prospecting

Advances have been made in new technologies – bio-prospecting – to explore the immense biological and chemical diversity in nature that has been difficult to access by natural methods. New tools are being used for actively screening for novel compounds produced by living organisms in different environments to evaluate their potential for use in medicine, agriculture and industry.

The microbial community represents the largest source of genetic diversity on the planet. A large number of new compounds of biological origin and mostly produced by the microbial community have been identified from different environmental sources. Studies of the compounds also reveal the large overlapping fractions of microbial genomes that could be used to disclose entire genomes of previously unknown micro-organisms even if present in very low

amounts. There is a strong demand to develop techniques for expression of the genetic information in various heterologous hosts to produce and characterize the new compounds of interest using DNA sequencing technologies. Bio-prospecting could also identify microbes that might serve as pathogens and provide an early warning for potential disease-causing agents. For drug development, agriculture and industry the information generated by bio-prospecting would be very significant in the near future.

New tools such as satellite mapping using high-resolution landscape datasets of pests and diseases, including insects and their behaviour, would greatly benefit eradicating invasive alien pests and diseases. The exploitation of new mobile computing, GPS, digital photography, telephone technologies, etc. provides precision and accuracy to information, which enables rapid transmission of key data for computation for better management of pest outbreaks. Conversely, bio-prospecting could be used as databank regarding the biological activity of dangerous pathogens and novel agents having severe effects and toxicity for humans, with the intent of introducing them into an immunologically naïve population. It could also impact on available measures of protection. The information on the novel compounds would also provide scope for modifications or synthesis by chemical means.

Bio-remediation

Bio-remediation technologies are being used worldwide to clear the environment of the harmful effects of the pollutants and convert them into useful products. Czech scientists, for example, have used bio-remediation technologies to detoxify mustard gas (yperite), using enzymatic catalysis with haloalkane dehalogenases. Haloalkane dehalogenases also provides useful applications in the production of alcohols to treat Alzheimer's disease or in biosensors to detect chemicals in the environment.

Non-lethal Biological Weapons (Nlbw)

Non-lethal weapons are intended to incapacitate personnel or materiel without visible injury or damage. NLBW involve potential military use of agents causing slow-onset infections and to create novel anti-animal or anti-plant BW that have consequences leading to economic losses.

Studies for creating NLBW are reported in the field of allergology, specifically the production of genetically engineered allergens. Recombinant allergens would include elements from the pollen of plants and epidermal and microbial allergens. Creation of highly productive recombinant strains will make it possible to produce large volumes of allergens in short periods of time. Another area for NLBW is reportedly based on the development of biological agents capable of pathologically acting directly on the genomes of people and animals without an infectious process. Pathology symptoms of such agents would have a lifelong nature, resemble hereditary diseases and be inherited from generation to generation, decreasing the viability of that hereditary line.

The social and economic consequences of outbreaks of animal and plant diseases are significant. The bubonic plague epidemic of 1994 in India, the outbreak of FMD in 2001 in Great Britain, epidemics of bird influenza H7N3 in Canada in 2004 and H7 virus in 2005 in North Korea, the 2003 epidemic of atypical pneumonia in Hong Kong, have been examples of huge economic loss. These epidemics also caused destruction of animals

and birds leading to reduction in tourism and a significant loss of exports. These financial implications become the source of motivation to create agents for use for prohibited purposes. This also increases significantly the danger of novel anti-animal and anti-plant BW being developed. Virtually all of the developments connected to agents of infectious disease can be realized not only for human pathogens, but also for animal and plant pathogens.

The Road To Weaponization

Some of the advanced technologies, tools and designs that lead to weaponization are:

- Rendering a vaccine ineffective;
- Conferring resistance to therapeutically useful antibiotics or antiviral agents in pathogenic organisms to produce an untreatable pathogen that is resistant to common antibiotics;
- Enhancing the virulence of a pathogen or rendering a non-pathogen virulent, to inflict increased human damage;
- Increasing the transmissibility of a pathogen so that it is more easily transmitted through a population;
- Altering the host range of a pathogen so that people would lose immunity to the disease;
- Enabling the evasion of diagnosis and/or detection by established methods so that in case of biological attack, there is delay in diagnosis and subsequent treatment;
- Undertaking genetic sequencing of pathogens to reconstruct a pathogen or develop a novel pathogen for deployment against a target population with no natural immunity;
- Synthesizing pathogenic micro-organisms to facilitate reconstruction of extinct or construction of novel pathogens;
- Enabling weaponization of a biological agent or toxin in making biological attacks more likely; and
- Experimentation with the smallpox virus so that it could be used in a biological attack.

Conclusion

Advances in biotechnology have brought in major changes in biology and life science areas through new techniques of genetic engineering and sequencing technologies, including rDNA technology that permits the transfer of genetic material between widely divergent species and changes in the character of micro-organisms. The increase in knowledge of many pathogenic species of micro-organisms, toxins and other biological agents and the continuing pace of developments in civilian-related biotechnology areas have further increased the possibilities for production and hostile use of biological agents, making BW an attractive option for governments seeking to acquire weapons of mass destruction. A BW programme can be hidden amidst dual-purpose industries.

Technologies like biotechnology, nanotechnology and information technology are converging in ways that will enable life processes to be manipulated with far-reaching implications and great potential for nefarious and disastrous outcomes. The tools discussed above interactively create unanticipated opportunities for these technologies to be used for the benefit of humanity and agriculture, while opening equal opportunities for their malicious use.

Interestingly, preparation of effective protective material against BW requires significant purification procedures and infrastructure for state-of-the-art produced vaccines, therapeutics, therapies, and prophylactic products; but the potential misuse of these technologies for the creation of effective BW does not require infrastructure of similar sophistication.

The implications of the advances in biological science and technology relevant to BTWC are being considered in relation to national implementation of Articles III, IV, VII and X. Monitoring and assessment of the scientific developments in biological sciences is considered important for the States Parties in their preparedness to counter outbreaks of disease, whether natural, accidental or deliberate, and in ensuring that national bio-security and bio-safety arrangements are up to date and effective enough to strengthen BTWC.