The misuse and malicious uses of the new biotechnologies

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John is the author of the article "The misuse and malicious uses of the new biotechnologies"; he proposes here a state of the art about the new biotechnologies and their possible applications.

(Abstract by Yves DUBUCQ, Managing Director ICI)

Several bacterial, viral agents and toxins can pose public health risk in the event of a bioterrorist or biological warfare attack. These agents, if used in any such attacks, can pose a difficult public health challenge and cause large number of casualties, and will be difficult to contain. The alleged use of biological agents is a serious problem and the risk of using these agents in a bioterrorist attack is increasing.

History dictates that bio-warfare goes back in time from the 14th century medieval siege of Kaffa (Feodosiya, Ukraine) by the Tatars who tossed dead or dying plague victims into the city and later (1763) when British troops provided blankets and linens used by smallpox victims to native Indians (Fort Pit, Ohio River Valley incident), all the way to most recent (1984) Rajhneeshee cult members who attempted to influence local elections in The Dalles (Oregon, USA) by contaminating salad bars with *Salmonella typhimurium* (food poisoning) and of course the 2001 anthrax letters global scare and certain incidents with ricin letters (2003 and 2013).

Biological warfare agents are microorganisms like virus, bacteria, fungi, protozoa or toxins produced by them, that give rise to diseases in man, animals or plants, when deliberately dispersed in an area (see the table 1).

These agents can cause large-scale mortality and morbidity, incapacitating a large number of people in the shortest possible time. The use of biological warfare (BW) agents can be covert or overt and they differ from conventional weapons by way of several unique properties. The effects of these agents are not instantaneous and require from few hours to weeks (incubation period) before the symptoms appear in the affected population (see the table 2).

These attacks require a release of small quantity of viable material, are capable of self-replication and can cause a disease outbreak in an area. Viruses are capable of

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	Agents	Disease	Route of infection	Possible release
Bacteria	*B. anthracis*	Anthrax	Aerosol	Spores
	Y. pestis	Plague	Aerosol	Vegetative cells
	B. melitensis	Brucellosis	Aerosol	Vegetative cells
	B. abortus			
	B. mallei	Glanders	Aerosol	Vegetative cells
	B. pseudomallei	Melioidosis	Aerosol	
Viruses		Smallpox	Aerosol	Virus particles
	Variola virus	Ebola hemorrhagic fever	Aerosol	Virus particles
	Ebola virus		Aerosol	
	Marburg virus	Marburg hemorrhagic fever	Ingestion food/water	Virus particles
Toxins	*C. botulinum*	Botulism	Food/water	Toxins
	S. aureus		Food/water	Toxins
	Ricin (plant)	Staph. Enterotoxin type B	Food/water	Toxins
	Trichothecene (fungus)	Ricin toxin		Toxins
		T2 toxin		Toxins

Table 1

replication only inside a living cell and are pathogenic to man, animals and plants. They consist of proteins and nucleic acids (DNA and RNA) and multiply and spread much faster. Bacteria are single-celled prokaryotic organisms and with a definite cell wall. Fungi are unicellular or multicellular, eukaryotic organisms and have no chlorophyll. Several fungal species are known to cause diseases in plants and few of them in humans as well (e.g. allergies). Toxins are secondary metabolites produced by bacteria, fungi, algae, plants, fishes, crustaceans and molluscs and are known to act in very low concentrations and can affect the functioning of cells

Developments in areas like physics, chemistry, 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.).

The Biological and Toxin Weapons Convention (BTWC) entails prohibition of the development, production, 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

Agent	Incubation period (days)	Person to person transmission	Morbidity/mortality if untreated
B. anthracis	1-5	No	High/high
Y. pestis	2-3	Yes	High/high
B. melitensis	5-60	No	High/low
B. abortus	3-7	No	High/low
B. mallei	3-7	No	High/low
B. pseudomallei	1-5	No	High/high
Variola virus	7-17	Yes	High/high
Viral hemorrhagic fevers	4-21	Yes	High/high

Table 2

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.

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.

Technical developments of major concern

- *Bio-defense:* Scientific and technological changes in detection, identification, diagnosis and protection provide increased capabilities to counter or protect against biological weapons.
- *Genetic modifications:* Considerable research on genetically modified live vaccines able to immunize simultaneously against multiple antigens while knowledge of the molecular basis of antigens led to antibody reagents of improved specificity.
- *Mechanism of action of micro-organisms:* Using molecular biology, mechanisms of virulence and infection have been identified, raising fears for deliberate manipulation of these mechanisms (e.g. via transferring genetic traits into naturally infectious micro-organisms or via altering their immunogenicity thus invalidating both vaccines and diagnostic methodologies).
- *Micro-biological developments:* Better knowledge of protein synthesis and assembly led to production and isolation of various proteins (*e.g. Escherichia coli; Yersinia spp.).*
- *Human Genome Project (HGP):* Identification and localization of genes causing hereditary diseases and simplification of the development of pharmaceutical drugs for treatment of hereditary diseases. HGP provides sufficient data on ethnic genetic differences between population groups, raising fears for future "ethnic bombs" (micro-organisms attacking known receptor sites or targeting DNA sequences inside cells by viral vectors). 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. A recent study in Taiwan has discovered that Severe Acute Respiratory Syndrome (SARS) can be associated with specific genetic profiles. Human Genome Project (HGP) – completed in 2003 – discovered all the estimated 20,000-25,000 human genes and determined the complete sequence of the three billion DNA subunits bases in the human genome.
- *Toxins and Regulators:* Large-scale extraction and production (lower cost/shorter time) of potent toxins, which until now were available only in minute quantities from immense amounts of natural biological materials. Understanding of bio-regulators and their effects, when present in abnormal concentrations. 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. 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.

Of equal importance are applications of biotechnology in directed molecular evolution such as genetic modification, recombinant technologies, proteomics, bio-informatics, and synthetic and systems biology.

Recombinant DNA (R-DNA) Technologies

Rapid and relatively inexpensive identification, characterization, mapping, manipulation and synthesis of genes and short strands of genetic material.

- *Genetic engineering:* Through reverse genetic engineering, researchers can introduce viral RNA into bacterial cells, where it can then be manipulated much more easily (e.g. poliovirus, yellow fever virus, H1N1, influenza A, rabies, corona viruses, H5N1).
- *DNA synthesis:* Generation of genetic sequences that specifically program cells for the expression of a given protein (e.g. poliovirus, 1918 influenza virus).
- *Genome sequencing:* Generation of genomics' data for a number of pathogenic micro-organisms leading to insights of their mode of action but also, if misused, could lead to conversion of a harmless organism into a pathogenic variant difficult to handle.

- *Fusion protein:* Insertion of a toxin in a protein, enabling it to identify and kill specific cells (cancer) but also if properly modified to kill cells essential to life.
- *Combinatorial chemistry:* Generation of huge libraries of synthetic compounds with diverse properties and screen them for activity against biological drug targets. If misused, can lead to compounds able to interact with physiological pathways leading to diseases.
- *High-Throughput (HTP) Technologies:* Related technologies (automated sequencing, mass spectrometry proteomics, transcriptomics, proteomics and metabolomics) generated a wealth of genetic information that might lead to identification of toxic compounds for both humans and animals.
- *Directed Drug Design:* Technique allowing to target a desired site and function, and design a drug with particular properties instead of screening via genetic libraries.
- *Synthetic Biology:* Combined technologies allow researchers to develop a registry of biological parts and essentially create tiny programmable computers from living organisms with various future applications (e.g. re-engineered bacterial proteins when tagged with TNT® are able to detect chemical or biological agent signatures or clean up environmental pollutants).
- *DNA Shuffling:* 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 but, if misused, generation of lethal bacteria and virus is possible.
- *Artificial synthesis of viruses:* Small viruses are being synthesized, based on availability of genetic code (e.g. poliovirus [2002]). 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.
- *RNA interference (RNAi/siRNA):* Technology suppressing cellular production of certain proteins in the physiological processes in order to halt undesirable processes or stimulate desirable ones of importance in therapeutic use (e.g. use of aptamers (short, single-stranded nucleic acids) and tadpoles (protein-DNA chimeras) for inhibition of blood clot formation or age-related ocular degenerative conditions. In theory, silencing of genes that function in innate immunity could lead to conditions that mimic, at least superficially, natural disease.

Genomics and Proteomics

Use of advanced sequencing technologies (*genomics*) led to mapping of the molecular signatures of the bioregulatory systems of the body and how these regulatory pathways respond to disease-induced disturbance. *Proteomics* can differentiate isolates or strains and greatly enhance our knowledge of host-pathogen interactions, protein-protein interactions, host response to infection and pathogenesis.

The information, which is critical as target for therapeutic and preventive intervention or manipulation, can also be used for a novel biological attack.

Vaccines and Therapies

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 diseases like HIV, hepatitis B and C and malaria, influenza viruses H1N5, H1N1, etc. Vaccines and anti-viral drugs of defence interest are also being developed, including anthrax, poxvirus infections, etc.

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 biological warfare (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.

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. 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

Designing, characterization, production and application of structures, devices and systems by controlling the shape and size of materials at nanometre scale. This technology combines biotechnology, synthetic biology and information technology to design molecular structures (e.g. new drugs) capable of performing a wide range of functions. 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 bio-weapons. 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 biological weapons'

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 Organization for Economic Cooperation and Development is working to promote international cooperation in the health and environmental safety-related aspects of manufactured nano-materials. Anthrax spores sent in the letters were coated with a very rare, high-tech glass polymer nano-material to avoid clumping thus easing aerosolization and consequent inhalation.

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, glycogen and chitin. Synthetic polymers (smart 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 (e.g. molecular imprinted polymers in combination with peptides used in diagnosis of mycotoxins).

Drug Delivery

A number of new matrices have been identified as carriers for prolonged and sustained delivery of drugs and pharmaceuticals (e.g. nano-emulsion technologies using large porous particles for drug delivery in the lungs). Alternatively, these techniques could potentially be used to develop highly efficient aerosol delivery systems for viruses and small bacteria, toxins or chemical compounds for bio-warfare/terrorism purposes.

Micro-encapsulation

Prolongation of the shelf life of micro-organisms or proteins in the body or the environment by coating or enclosing them in a biopolymer capsule. This knowledge might enhance 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

Aerosol technology is among the widely accepted delivery vehicles to deliver biologically active organisms or compounds, including therapeutic molecules, to target structures (e.g. via use of micro-encapsulation techniques or bacterial plasmids). Evolving technologies raise concerns about the delivery of bio-regulators and other toxic substances through use of aerosol technology.

Plant Pests and Diseases

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. 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.

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 (i.e. *Bacillus thuringiensis* transgenic crops). 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 toxins harmful to crops, animals and to some extent in humans.

Bio-prospecting

Technologies exploring the immense biological and chemical diversity in nature that has been difficult to access by natural methods.

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 provide 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. 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 *foot-and-mouth disease* 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 biological weapons 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 loose 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.

In conclusion

There is no doubt that biotechnology have already made gigantic steps allowing a better understanding of how human, animal and plant organisms function. More and highly sophisticated technologies will surely follow. But this progress is not immune to malicious actions leading to overt and covert destruction and disruption of modern societies. It is mainly depending on humans to decide if they will prefer peace and progress for good or choose evil that will eventually end life in our already suffering planet.

"Pandora's Box had been opened and monsters had come out. But there had been something hidden at the bottom of Pandora's Box. Something wonderful : Hope!" (Lisa Marie Rice (author), "*Breaking Danger*").

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