

## Possible Terrorist Use of Modern Biotechnology Techniques

---

RAYMOND A. ZILINSKAS

Conference on Biosecurity and Bioterrorism  
Istituto Diplomatico "Mario Toscano"  
Villa Madama, Rome, Italy  
*September 18-19, 2000*

## INTRODUCTION

In early 1999, the Center for Counterproliferation Research at the National Defense University (NDU) began a collaboration with the Center for Nonproliferation Studies, Monterey Institute of International Studies (MIIS) to assess the likely impact of recent and anticipated advances in biotechnology on the ability of terrorists to acquire and employ biological agents.<sup>1</sup>

The forecasting method selected for this project was to use a focus group. A focus group consists of experts brought together to consider a series of issues needed to address the subject of concern. The focus group approach is useful for identifying areas of consensus or disagreement on presented issues. The NDU/MIIS focus group, which included both natural and social scientists, possesses a wide range of expertise. However, most of its members are researchers working the biological sciences; they are affiliated with academic institutions, industry, and government agencies (see Annex 1).

The focus group was asked to consider the possibilities offered by the advanced techniques of biotechnology to terrorist or criminal groups (hereafter combined under the single heading of "terrorists") in the next five years, i.e., up to 2005, to the weaponization of pathogens and toxins. Specifically, the focus group was tasked to:

- 1) analyze newly developed and emerging biotechnology techniques in terms of their utility in research and development (R.&D) aiming to produce microorganisms of terrorist utility.<sup>2</sup>
- 2) determine the level of training required by persons who would employ these techniques and the equipment and facilities they would require to do their work.
- 3) concentrate on possible applications directed against human populations.<sup>3</sup>

A draft report containing the findings of the focus group has been written; it currently is being reviewed by outside experts. We expect to incorporate the suggestions by these experts and issue a final report by September 2000. Due to the sensitive nature of some of its descriptions and findings, it will be distributed only to government agencies. A less sensitive version of the report will be published later.

For the purposes of this meeting at Dartmouth, I abstract focus group findings in three areas: (1) attributes of microorganisms that a bioweaponeer would find profitable to enhance; (2) advanced biotechnologies that may be used for that purpose, and (3) main conclusions and recommendations made by the focus group. I end with a short paragraph that discusses two issues that flow from our work and that the Dartmouth conference might consider.

### I. WEAPONIZATION OF MICROORGANISMS

The five attributes that characterize a "perfect" military biological warfare (BW) agent have already been identified.<sup>4</sup> They are as follows:

- 4) High virulence coupled with high host specificity;
- 5) High degree of controllability;
- 6) High degree of resistance to adverse environmental forces;
- 7) Lack of timely countermeasures to the attacked population;
- 8) Ability to camouflage the BW agent with relative ease.

Some of these attributes might not be so important for BW agents that will be applied for terrorist purposes. For example, an apocalyptic terrorist group might be unconcerned whether or not the agents it uses can be controlled after release. Nevertheless, these criteria served as a useful starting point for our considerations of the scientific objectives scientists working for bioterrorists may have when applying modern bioscience and biotechnology to weaponize microorganisms. Thus, to develop perfect BW agents, modern biotechnology techniques may be applied to enhance any or all of eight characteristics or traits of microorganisms – hardiness, resistance, infectiousness, pathogenicity, specificity, detection avoidance, senescence, and the viable but non-culturable state.

### **A. Hardiness**

Hardiness refers to the ability of a microorganism or a bacterial or fungal spore to survive being enclosed in a storage container or munition and, after release onto the target, survive physical and chemical stresses encountered in the open environment. A scientist might attempt to enhance the hardiness of bacteria, fungi, and viruses in two ways. First, he could try to enhance the organism's ability to resist desiccation, withstand ultraviolet (UV) radiation from the sun, and survive decontamination procedures. If successful, the BW agent would survive longer after release, thereby increasing its potential for causing casualties. Second, an attempt may be made to stabilize genetically determined traits, such as virulence, in the weaponized agent. If this was done, the agents constituting payloads of biological weapons would have a longer shelf life, thus lessening the need of continually reload them with freshly produced agents.

With the germinating cells of bacteria, hardiness depends mostly on the bacterium's repair mechanism; i.e., the quickness and thoroughness with which the bacterium's genetic makeup is able to repair damage caused by stressors to its cell wall, chromosome, and other structures. However, due to inadequate scientific knowledge about the genetic control over repair mechanisms in bacteria and limits to the ability of scientists to transfer multigene constructs from one organism to another, the locus group believes that no scientist will be able to genetically increase the hardiness of a bacterial species before 2005.

In relation to bacterial spores, such as those of *Bacillus anthracis*, nature has made them hardy for the specific purpose of tolerating environmental stresses. In the next five years, science probably can do nothing to improve on nature with regard to enhancing the hardiness of bacterial spores.

Even less is known about the repair mechanisms of fungi than of bacteria, therefore, no one is likely to be in a position to apply molecular biology techniques for the purpose of increasing the hardiness of these organisms before 2005.

Some viruses, such as the smallpox virus, are exceedingly hardy, being able to withstand desiccation for many hours. But most viruses die within minutes after release into the open environment due to desiccation. It appears that the hardiness of viruses depends mostly on the chemical structure of their outer coat. While it is possible to attempt to alter the outer coat of some viruses to change their presentation (see below), there is insufficient knowledge on how to do so to achieve greater hardiness. Most likely, if an attempt to do so were made, other traits of the modified virus would be degraded, such as invasiveness and virulence. For these reasons, there is little or no possibility of scientists, even when applying sophisticated biotechnology techniques, being able to enhance the hardiness of viruses in the next five years.

### **B. Resistance**

Resistance refers to the ability of a microorganism to defeat the actions of therapeutic drugs, such as antibiotics, and preventives, such as vaccines.

The means by which different microorganisms are able to resist drugs and preventives vary considerably from type to type. In regards to bacteria, a scientist might attempt to develop strains that are resistant to antibiotics used by the target population; if virus, the aim could be to develop viral strains that are unaffected by the enemy's antiviral therapeutic drugs; or if a fungus, an effort could be made to develop a strain that resists fungicides and antifungals. The advantage to the bioterrorist of using highly resistant strains in an attack would be greater casualty generation and higher lethality among those attacked.

Imbuing a bacterial strain with antibiotic resistance is no longer a substantial scientific challenge. Many plasmids with resistance markers are available in ordinary bacterial strains; these may be moved into new hosts using either classical or molecular biology techniques. Having stated this, it must also be made clear that although the development of antibiotic resistant bacterial strains is technically not so difficult, this does not guarantee that the altered strains will be better suited for weapons use than their less antibiotic-resistant relatives. The reason is that the newly developed antibiotic resistant strains may evidence pleiotropic effects (unwanted and unplanned characteristics); i.e., the newly engineered strains will possess not only the desired characteristic of antibiotic resistance, it also will manifest additional but unwanted characteristics that will make it unsuitable for weapons purposes, such as less virulence or hardiness (or both). Pleiotropy is discussed in more detail below.

### ***C. Infectiousness***

Infection is the process whereby microorganisms invade and establish themselves within the body of a host. Whether or not a microorganism is able to infect a host depends on the outcome of a series of complex interactions between the invader and the host. The bioterrorist scientist can attempt to enhance the invasive abilities of microorganisms being developed for BW. In general, pathogens possess hydrolytic enzymes that destroy lipids and proteins. Since precisely these chemicals constitute the membranes and walls of the host's cells, they become the targets for a pathogen's attack. Scientists thus may imbue a pathogen with the ability to secrete enzymes that act to circumvent antibodies secreted by skin cells such as Immunoglobulin A (IgA). Another approach would be for a scientist to attempt to enhance the ability of bacterial cells to adhere to the walls of the respiratory or intestinal tracts. In immunocompetent hosts, these tracts are protected by being continuously flushed by fluids and by cells lining the tracts secreting protective substances such as mucus and antibodies. To overcome these defenses, pathogenic bacteria produce special proteins, adhesins or receptors, which bind specifically to receptors (proteins on both interacting cells may be called "receptors") located on host cells. Adhesins and ligands are located either on the bacterial cell wall or on structures that protrude from the cell wall such as pili. Since a substantial amount of information is available in the scientific literature about these substances and how they are produced by pathogens it is possible that scientists could use this information to design projects aiming to imbue pathogens that normally do not produce adhesins with the capability to do so, and enable pathogens to secrete viscous substances, such as alginate capsule and polysaccharide slime, thereby increasing their ability to adhere to host cells.

All mammals are able to produce a large array of defensive peptides that act to destroy invading pathogens. Two types of peptides, defensins and cathelicidins, in particular are vital to a mammal's defense. Alpha defensins are found in the blood and intestinal epithelia, while beta defensins defend the kidneys, urogenital tract, and skin. If a weapons scientist were able to design a pathogen that possesses proteinases with the ability to destroy these peptides, it may well become a powerful BW agent.

There also might be possibilities for increasing the infection capabilities of viruses. Before being able to initiate infection, viruses must attach to an appropriate receptor on the prospective host's body cells. For example, the human immunodeficiency virus (HIV) produces a special protein (gp 120) that attach to receptors on the T lymphocytes (a type of cell that is part of the body's immunodefense system), thus allowing the virus to enter these cells whose

normal function is to destroy invading microorganisms. Similarly, the influenza virus uses a protein called hemagglutinin as a type of adhesin to attach to a receptor on respiratory tract cells. Using information that has been published about viral pathogens, scientists can attempt research that aims to alter the genetic makeup of a virus so it can attach more efficiently to receptors or to receptors that it normally could not, of host cells.

Practically speaking, however, there is a little information about how microorganisms penetrate skin. Therefore, no one would be in a position to enhance this particular attribute in a pathogen or transfer the gene (or genes) that controls it from one organism to another. More is known about adhesins and their genetic control. However, it is not known whether the gene controlling adhesion in one microorganism would be expressed in another microorganism. Further, even if such a gene was expressed, it is possible that the gene transfer would result in pleiotropic effects. Therefore, laboratories working for terrorists probably would find research in this area as not worthwhile.

#### ***D. Pathogenicity***

Pathogenicity refers to the ability of the pathogen, once established within the host, to traverse the bloodstream or lymphatics, evade the intrinsic defenses of the host, enter target tissues of the host, and exert such damage that either injures or kills the host. In general, a pathogen that acts quickly do cause severe damage is considered to be virulent. For example, the smallpox virus and the bacillus causing anthrax are classified as virulent pathogens.

The successful invaders ability to damage the host depends mainly on the operation of a number of virulence factors working in unison to cause damage to the host. It would appear, therefore, that if a scientist was able to add virulence factors to a microorganism being developed for BW, or could enhance a pathogens intrinsic virulence factors so they would work more efficiently, the modified microorganism or pathogen would make a better BW agent.

Virulence factors may be grouped under one of three more general headings- local effects, distant effects, and evasion of host defenses.

- 9) Local effects. After taking up residence in a hosts tissue, some pathogens secrete enzymes and other substances, such as coagulases, kinases, lecithinases, and proteases, which break down the host's cells and intracellular matrices located proximal to the infectious foci, for example, the so-called "flesh-eating bacteria are strains of Group A *Streptococcus* possessing virulence factors facilitating rapidly progressing subcutaneous infection.
- 10) Distant effects. Some virulence factors are released by the established pathogens and are carried by the hosts circulatory or lymphatic system to distantly located organs. Among these types of virulence factors, toxins may be of highest importance. Many bacterial pathogens are able to secrete toxins; once these have been liberated and circulate throughout the body of the body, they produce fever, shock, and death.
- 11) Evasion of host defenses. Pathogens have evolved numerous strategies to evade host defenses and to utilize substances produced by the host for their own purposes. Thus, many pathogenic bacteria are able to secrete special proteins, called siderophores, which can remove iron from the hosts carrier proteins and make it available to the bacterial cell Some pathogens, such as *Streptococcus pneumoniae* and *Cryptococcus neoformans*, produce a glycocalyx capsule that protects the vegetative cell from phagocytosis. There also are species of *Staphylococcus* and *Streptococcus* that secrete leukocidins capable of destroying the host's leukocytes and hemolysin and lysing red blood cells. Some bacteria (such as rickettsias) and viruses (such as HIV

and herpes virus) hide within the host's cells, thus evading the host's immune response.

Most virulence factors are proteins secreted by the invading pathogen that act by destroying normal host functions, of which the pathogen then takes advantage. It would appear that the genes controlling the production of some of these proteins would not be difficult to identify and transfer to microorganisms being developed for BW purposes. Quite probably, scientists attempting to weaponize bacteria and fungi would have a plethora of choices as to which virulence factors he could use. Further, although viruses are unable to directly secrete proteins, some can be imbued with genes that code for protein production and are expressed when the virus takes over the host cell. For example, recently scientists were able to insert genes, and appropriate promoters, that code for scorpion neurotoxins into a virus used for insect control to improve their insecticidal effectiveness.<sup>5</sup> It would appear that a similar approach could be used for the purpose of developing more pathogenic viruses for purpose of BW.

It would be fairly easy for an appropriately trained junior scientist or scientist to identify genes coding for many of the well-characterized virulence factors and to transfer these genes from the cells of one bacterial species to another. This is particularly so when a single gene codes for a single protein of importance, such as an adhesin or a toxin. Further, it is well recognized that in some bacterial species, such as *Escherichia* species and *Vibrio* species, very small differences in the organisms genome, for example, the absence or presence of a single gene, will determine whether the strain is pathogenic or nonpathogenic. Single genes such as these are easily transferable from one cell to another. It therefore can be concluded that it would be feasible for a bioterrorist scientist to employ the advanced techniques of biotechnology in an effort to enhance the pathogenic potential of well-studied bacterial species through the transfer of genes coding for virulence factors.

In consideration of fungi, much less is known about their pathogenic mechanisms and modes of action than with bacteria. It is therefore highly doubtful that someone will be able to enhance the pathogenicity of fungi in the next five years.

Much less known about viral virulence factors than bacterial and fungal virulence factors. For this reason, it is not probable that anyone will be in a position to deliberately affect viral virulence factors before 2005.

Similar qualifications to those stated at the end of Section B above must also be noted here. While it is not a technically difficult for an appropriately trained junior scientist or scientist to transfer a gene coding for a virulence factor from one bacterium to another, the newly transformed bacterium might exhibit pleiotropic effects that will render it less suitable for weapons purposes than the original strain

### ***E Specificity***

Specificity refers to a pathogen's propensity to prefer a specific host. A scientist working for bioterrorists might find it useful to either to increase a pathogen's preference for a specified target population or to decrease the pathogen's ability to attack populations other than the target population. By doing so, the probability of a biological weapon causing collateral damage is decreased, thus increasing the bioterrorist's ability to control the weapon.

Host preferences among pathogens vary widely. At the one end of the scale, some species of viruses (for example, certain animal influenza viruses) and bacteria (for example, *Mycobacterium leprae*) tend to be species specific. At the other end of the scale, there are many bacterial and fungal strains that attack more than one animal or plant species. For example, some subspecies of the bacterial species *Pseudomonas aeruginosa* can cause disease in every known kind of animal, be it vertebrate or invertebrate, warm blooded or cold-blooded. Although viruses tend to have a narrow host range, some RNA viruses are capable of using pathways

outside their usual host range. For example, the foot-and-mouth virus, which is commonly thought of as only being able to attack cloven-footed animals, recently has been shown to be able to infect and propagate in human cells

The issue of specificity has become a subject of intense interest during the last few years. There are two reasons why. First, the Human Genome Project (HGP) will have mapped the entire human genome by 2003 and this information will, to all appearances, be easily accessible to anyone possessing a computer equipped with a modem. One of the implications of this development is that scientists might be able to utilize information generated by the HGP to identify genetic markers specific to certain populations and to perform research for the purpose of developing pathogens or antigens that will preferentially harm individuals possessing these markers.<sup>6</sup>

Second, a host of smaller projects are being undertaken in parallel to the HGP, the goals of which are to map the genomes of viruses, bacteria, fungi, insects, and worms. By 2000, the complete genomes of about 13 pathogens had been fully sequenced, and another 60 pathogen genomes were well on the way on being characterized.<sup>7</sup> It is reasonable to assume that over a hundred pathogen genomes will have been published by 2005. From the information generated so far by whole genome research, it is already possible to identify certain genetic characteristics of microorganisms that characterize them as pathogens. The possibility, then, is that scientists may use this information to undertake research with the aim of transforming non-pathogens to frank pathogens or, even, creating truly new pathogens.

The biological relationships between hosts and pathogens, be they bacteria, fungi, or viruses, are exceedingly complex, having evolved over thousands or more years. While research on the genetic basis governing some host-pathogen relationships is beginning to produce findings, knowledge about these relationships is still rudimentary. It therefore is the sense of the focus group that it is most unlikely that even the most qualified scientist would be able to enhance the specificity of any type of pathogenic microorganism before 2005

### ***F. Detection Avoidance***

There are two types of detection avoidance. First, it could be the deliberate altering of properties possessed by well-characterized BW agents, such as engineering it to express surface antigens it normally would not express. If so, the target population, using existing methods, would have problems with detecting and identifying the modified form of pathogen. Second, an organism could be deliberately altered to defeat the immunological defense systems of a target population.

In reference to the first type of detection avoidance, all known biological threat agents have been characterized to the point that were one of them to be used in an attack, it would be identified within a short time so appropriate treatment would be administered to exposed populations. Thus, if bacteria are used in an attack, antibiotics would be administered to exposed persons; if viruses were used, it might be possible to administer anti-viral medicines and, if the virus is contagious, institute quarantine and initiate a vaccination campaign to stop further spread. To defeat these defensive measures, a bioterrorist scientist might endeavor to alter a specified organism's antigen presentation, thereby making it difficult for defenders to identify the BW agent through the use of existing detection methods. By doing so, it is likely that the victims of a biological attack would receive delayed, sub-optimal, or erroneous treatment, or a vaccination campaign might not be undertaken in a timely manner.

To develop a bacterial strain that defeats detection by clinical methods, a scientist could attempt to manipulate one or a few genes that control bacterial metabolism or the production of proteins constituting the bacterium's cell wall. By altering a bacterium's metabolic properties, the work of the clinical laboratory to identify the bacterium is made more difficult. In regards to altering the bacterial cell wall, if this were done the modified organism's antigenic presentation would be sufficiently changed to confuse detection methods usually employed in

the clinical laboratory to identify organisms to the level of species, such as the polymerase chain reaction (PCR)<sup>8</sup> and mass spectrometry. Similarly, the modified organism might avoid detection by field investigators employing array kits designed to quickly identify any of a number of biological threat agents

The second type of detection avoidance refers to circumventing primed immunodefense systems of the target population. Human populations of industrialized nations are routinely vaccinated against many common diseases. Shortly after being vaccinated, the vaccinated individuals develop antibodies that most often are able to defeat the pathogens against which vaccines have been developed and administered. In other words, the immunological defenses of vaccinated populations are primed to meet the threat of certain infectious diseases. To defeat this type of defense, a scientist working for terrorists could attempt to genetically engineering a classical threat agent so that its genetically modified form is antigenically different from the parent. If he were successful, the antibodies constituting part of the target population's immunodefenses would not recognize the new antigenic presentation, leaving the host vulnerable to infection by the modified form. In bacteria altering the cell wall, as described above, could do this. With viral species, the scientist could attempt to change the viral coat. Many viruses, especially RNA viruses such as influenza viruses, mutate frequently in nature, in the process changing their antigenic presentation. Research has been, and is being, conducted for the purpose of clarifying how viruses accomplish this; some findings of this research have been published. A scientist might be able to utilize published information in research that aimed to change the antigenic presentation of viruses being developed for weapons use.

Of the two types of detection avoidance, the first type, altering a BW agents presentation, could be done relatively easily by someone possessing expertise of the level of junior scientist or scientist. However, if genetic manipulations were done on an organism for this purpose, to, for example, alter a cell wall or viral coat, it is almost certain that the manipulated organisms would exhibit pleiotropic effects, such as the manipulated organisms ending up with a weakened external structure. As has been explained above, the research needed to develop a useful BW agent with altered presentation therefore would be risky, probably best done by a national program. It also appears dubious that this kind of research would bring significant added value to a BW agent; therefore it hardly would be worthwhile for a terrorist organization to support it.

The focus group believes that research to accomplish the second type of avoidance detection, that of circumventing primed immunodefenses of a target population, is not likely to be done before 2005. The main reason for this finding is that before such research could be undertaken, difficult field research would have to be done by the future attacker to investigate the immunological status of a target population to be attacked. This would take a long time to complete and probably would in any case not produce findings of sufficient completeness to design an offensive project to develop a BW agent uniquely suited to take advantage of weaknesses or defects found in the target population's immunological defenses.

### ***G. Senescence***

Theoretically, microorganisms can live forever. Thus, bacteria and fungi keep subdividing *ad infinitum* as long as the supply of nutrients is sufficient and their wastes do not accumulate to a toxic concentration, while viruses will survive as long as they can find new host cells that can be programmed to assemble new virions.

Under most circumstances, a bioterrorist can be expected to prefer to have limits on the scope and length of a biological attack he orchestrates. If a limited attack was possible, the enemy would suffer, but the probability of the attack affecting friendly or neutral populations would be lessened. One way of limiting the time and/or extent of attack might be by deliberate senescence; i.e., to genetically engineer BW agents so they die on cue.

During the last five years scientists have developed sophisticated mechanisms for ensuring that certain genetically engineered microorganisms (GEMs) do not survive after having performed a specified task. To this end, scientists have designed genetic constructs that program the death of the cell into which they are placed under specified conditions. Such constructs, called suicide constructs, typically include a gene that codes for production of a toxin lethal to the host cell and a promoter sequence that activates the toxin gene in response to a precise signal, such as a temperature change or the presence or absence of a specific chemical or nutrient. For example, a recently developed suicide construct allows cells of a biodegrading strain of *Pseudomonas putida* to survive only in the presence of certain aromatic hydrocarbons it has been engineered to degrade.

An imaginative weapons scientist might be able to develop a genetically engineered contagious bacterium or fungus useful for BW that contains a suicide construct. The suicide construct would be designed so that it becomes activated when, for example, the ambient temperature exceeds or falls below a specified range, or when a certain chemical is encountered, or when a certain chemical is not present. More difficult to accomplish, a scientist working for bioterrorists might attempt to develop a suicide construct that activates in a bacterium after it has undergone a certain number of cell divisions or in a virus after it has passed through host cells a certain number of times. If this were done, it would be possible to use contagious pathogens for BW purposes in a controlled manner.

It was the sense of the focus group that although more is becoming known about the natural senescence of microorganisms and substantial work has been done to design clever suicide constructs, there is still much to learn before it would be possible for anyone to develop a BW agent with controlled senescence. This almost certainly could not be done before 2005.

#### ***H. The Viable but Non-culturable State***

Many types of marine bacteria, including *Vibrio cholerae* (the causative organism of cholera) spend much of the life in a state called viable but non-culturable (VBNC) state; i.e., the bacteria are viable but are in a dormant state and cannot be cultured employing standard microbiological technology. Although it is not yet clear why bacteria enter the VBNC state, it has been determined that the VBNC phenomenon is under genetic control. Much research is being undertaken with the aim of clarifying the VBNC phenomenon, some important findings have already been published.

The possibility is that a scientist might try to utilize this information to develop pathogens uniquely suited for biological attacks. For example, if a scientist knew how to cause *Vibrio cholerae* to enter the VBNC state, he could attempt to suspend a large number of the dormant pathogens in the water filling the bilges of a ship. The ship could be dispatched to the port of the enemy, where it secretly would empty its bilges. At some time determined by, for example, a rise in water temperature or the appearance of certain nutrients in the water, the dormant organisms would revert to their active, pathological state. Anyone consuming seafood, such as fish and shellfish, taken from the area contaminated by the active vibrio would risk contracting cholera.

The sense of the focus group is that a clever junior scientist or scientist would be able to manipulate the VBNC state in a few well characterized food and water borne agents for purposes of crime or terrorism. With today's techniques it would be possible, for example, to induce the VBNC state in *Vibrio cholerae* by withholding certain metabolites. The bioterrorist then could contaminate food or beverage with the unculturable vibrios. The metabolite required to bring the organisms out of the VBNC state could be added to, for example, salad dressing. When the salad dressing is applied to salad, the vibrios would revert to their normal pathogenic state, sickening all who had consumed the combination of salad and salad dressing.

The focus group also considered why a terrorist would go through those steps to cause illness among people rather than using an active pathogen directly. There is no ready answer,

but I might be that a deranged scientist would do so for reasons that are obscure to us or for the satisfaction of overcoming a technical challenge.

## II. ADVANCED BIOTECHNOLOGY AND MICROORGANISM WEAPONIZATION

The focus group analyzed three sets of advanced biotechnology techniques that appeared to be of most immediate use to those who would attempt to weaponize pathogens: DNA technologies, genetic and protein engineering, and cell and tissue culture.

### *A. DNA Technologies*

Of the DNA technologies, three merit consideration; gene machines, sequence banks for proteins and nucleic acids, and the ongoing project to map the human genome.

#### i. Gene Machines

A gene is a section of DNA that codes for a defined biochemical function, usually the production of a protein. Instead of cloning genes or assembling them from cloned fragments of DNA, scientists can synthesize genes by using a gene machine (or DNA synthesizer). However, because many genes are longer than can be easily synthesized, a gene usually is assembled from several oligonucleotides (oligonucleotides are DNA molecules 01 100 bases or less). A scientist might use a gene machine to assemble genes that code for the production of desired proteins, such as toxins and virulence factors.

ii. Sequence Banks for Proteins and Nucleic Acids Bioinformatics is the use and organization of information of biological interest.

Much of bioinformatics is concerned with organizing databases that contain this information and of making that information available to those who need it. An enormous amount of data are available on DNA sequences, protein sequences, the human genome, enzymes, and other subjects from organizations such as National Center for Biotechnology Information, the DNA Data Bank of Japan, the Genome Database (GDB) of the Human Genome Project (HGP), and the European Molecular Biology Laboratory. Any scientist who has access to a computer equipped with a modem can access these databases and secure information on genes and proteins of BW Interest. Further, a large number of computer software programs have been designed to help scientists utilize the enormous amount of information available for purposes such as designing macromolecules, including toxins.

#### iii. Map of the Human Genome

When the HGP ends in 2003 (or sooner), the 80,000 to 100,000 genes that constitute the human genome will have been mapped and this information will be entered into the GDB<sup>9</sup>. Already data generated by the HGP has given rise to a new scientific field called genomic information technology, but more commonly known as "functional genomics." Functional genomics attempts to correlate the activity of a gene with specific activities, such as protein production, disease processes, signaling between body cells, and many others. It has been aptly stated "The fundamental strategy in a functional genomics approach is to expand the scope of biological investigation from studying single genes or proteins to studying all genes or proteins at once in a systematic fashion." Using functional genomics, scientists are beginning to clarify how genes interact with one another. Most likely, there are many interactions between genes, and between genes and the environment, which control the molecular basis of health and disease.

Scientists working for or on the behest of bioterrorists can, like scientists performing licit research, easily access the GDB. They then might apply functional genomics to identify genetic markers possessed by populations of interest to them. There has been the occasional

article in the arms control literature about ethnic weapons (see above), but such ideas have seemed farfetched until now when the HGP is close to achieving its objective. The question is, can information generated by the HGP be used to design biological weapons that selectively effect a chosen population? This question is discussed below.

### ***B. Genetic and Protein Engineering***

Genetic engineering is a general term for the genetic manipulation or genetic modification of animals, plants, and microorganisms. The oldest, most commonly used, and best-known genetic engineering technique is gene cloning (or splicing), which produces recombinant DNA (rDNA). Simply put, rDNA techniques allow scientists to isolate a gene from the many genes that constitute an organism's genome, and amplify it so it can be examined, altered, and/or emplaced in the genome of another organism. The final step, that is of inserting a gene taken from one organism into another, can be performed using any one of a number of methods, including transfection, transduction, and electroporation, so there is good reason to believe that similar difficulties are going to beset scientists developing genetically engineered bacteria for terrorist purposes.

Since it is possible, or even likely, that any genetic manipulation of a pathogen done for the purpose of increasing its value as a weapon will also imbue the manipulated organism with unwanted characteristics, the modified organism would have to be field tested before its weapons value can be guaranteed. This kind of activity is not easy to do and, further, outsiders might detect it. To test for virulence, for example, the developer of the agent probably would have to use animal models or, covertly, human beings, before the agent's increased value for weapons use can be ascertained. If a pleiotropic effect is noted that decreases the modified organism's value for weapons use, further research and experimentation must be done by the developer to remove the unwanted pleiotropic effect while retaining the modified organism's added properties. The implication of these uncertainties is that genetic engineering research undertaken for the purpose of enhancing a microorganism's utility for weapons use is risky for two reasons. First, it might fail. Second, even if an organism with apparently enhanced properties were developed, there is a substantial possibility that pleiotropic effects will become manifest in the modified organism, necessitating further research, development, and testing to remove them. It could take a long time and considerable effort before an organism exhibiting superior qualities for weaponization was developed; conversely, the entire effort might in the end fail.

### III. CONCLUDING THOUGHTS

The focus group established by the NDU and MIIS grappled with the question of when we can expect that results from applications generated by advanced biotechnology will become realized for terrorist purposes. It concluded that by 2005, few such applications are likely to appear. These few pertain to scientists working for bioterrorists who would be able to develop bacterial pathogens possessing increased resistance against antibiotics, being imbued with added virulence factors, having altered antigenic presentation and, perhaps, being made more controllable through the VBNC phenomenon. However, due to possible pleiotropic effects, none of these properties will necessarily result immediately in the modified organism becoming more suitable for weapons use.

Keeping these uncertainties in mind, it is the sense of the focus group that two types of bioterrorists are in the best position to apply the advanced techniques of biotechnology in research to enhance microorganisms for purposes of BW. The first type consists of states possessing BW programs and supporting international terrorist groups. Since these state programs can be assumed to be staffed with qualified technicians and scientists, well funded, and designed to operate for the long-term, they are best placed to undertake the type of risky

R&D described above and to perform adequate field testing that would ascertain the newly developed agent's value for weapons use.

While it is impossible to forecast exact reasons why a nation would want to equip its dependent terrorist group with weapons whose effects depend on genetically engineered weapons, two possible reasons are: (1) Just before the government of the terrorist-supporting nation initiates general hostilities against an enemy nation, it could order its dependent terrorist group to use biological weapons against that nation for the purpose of killing its leaders, demoralizing its military force, and spreading panic and confusion among its civilian population. It used in this kind of attack, the biological weapon equipped with the enhanced organism could be expected to cause a higher number of casualties than a classical BW agent, (2) The terrorist-supporting nation may feel that it is not strong enough to fight an enemy nation using conventional arms, but nevertheless wants to harm the enemy nation for reasons of revenge, jealousy, etc. For example, governments of nations such as Cuba and Iraq have indicated strong grievances against the U.S., but are too weak to seek recourse by traditional military means. Knowing how powerful and damaging biological weapons are, they might vent their frustration by ordering their dependent terrorist group to carry out a biological attack. If done correctly, not only would the attack cause terrible damage and harm, but also there would be little risk of the responsible party being identified. In this type of attack, the genetically engineered organism might be designed to cause high casualty rates and to be difficult to detect and identify.

The second type is the disgruntled or deranged scientist who works in a well-equipped clinical microbiology laboratory or academic laboratory involved in some aspect of microbiological research. This kind of person can be expected to have the knowledge, patience, and resources needed to undertake and complete the research he perceives is needed to accomplish his objectives and to do the testing necessary to ascertain the newly developed agent's value for weapons use. The disgruntled scientist might wish to get back at someone or some organization and would use a new strain of microorganism developed by himself to do so. This organism might be more deadly, or more difficult to treat, or have specific effects. The deranged scientist might undertake to develop a particularly clever and vicious organism just to demonstrate that he can do it. Lest someone believes that this seems farfetched, he should regard present-day computer hackers. Some of them demonstrate how clever they are by designing and dispersing destructive computer viruses; the proof of their cleverness is the amount of damage creations cause to people who have never harmed them in any way.

While recognizing that it is a chancy endeavor to predict developments that might occur during 2005 and 2009, certain research currently being done could give rise to findings applicable to a much greater degree than formerly in the development of BW agents. The implications of research for BW particular needs watching in six areas: (1) human functional genomics; (2) bacterial functional genomics, (3) pathogenicity islands; (4) synthetic viruses; (5) synthetic mycoplasmas; and (6) fusion proteins. In view of the rapid advances that we have seen in these areas during the last few years, assessments such as the one done here should be repeated every two years.

#### IV. POSSIBLE ISSUES FOR DISCUSSION AT THE DARTMOUTH CONFERENCE

As far as I knew, the problem of pleiotropic effects has never before been discussed in meetings addressing bioscientific advances that may be used for purposes of biological warfare and weaponry. There have been many statements made on how genetic engineering can be used to enhance the pathogenic properties of microorganisms, but not on the problems that might accompany such manipulations. If these problems will turn out to be minor, then advanced biotechnologies hold real promise to those who wish to use them for weapons purposes. If the problems are likely to be of a major nature, it is one less aspect of biological weapons development for us to worry about. Which is it?

Close related to the foregoing is the matter of “field testing”. By far, new products developed for peaceful purposes are extensively tested in the laboratory and the field before they are marketed. Would the scientists and technicians working for terrorists have the time, resources, and patience needed to perform testing before their new creations are unleashed? If not, there is a substantial possibility that their creations will fail when used in attacks. How do we address failed attacks? Indeed, how do we determine whether a failed attack has taken place? As far as I am aware, this issue has never been addressed.

#### Annex 1: Members of the NDU/MIIS Focus Group

Dr. Ken Alibek  
Dr. Seth Carus (co-chair)  
Dr. Rita R. Colwell  
Dr. Rolf A. Deininger  
Dr. David Franz  
Dr. Donald A. Henderson  
Dr. Raymond Kaempfer  
Dr. Scott Lillibridge  
Mr. Milton Leitenberg  
Dr. Lawrence Loomis  
Dr. Charles E. Main  
Dr. Allan J. Mohr  
Dr. Steven S. Morse  
Dr. Drew Richardson  
Mr. Brad Roberts  
Mr. Masaaki Sugishima  
Dr. Jurgen Von Bredow  
Dr. Mark L. Wheelis  
Dr. Raymond A. Zilinskas (co-chair)

#### Endnotes and References

1. For purposes of this study, biological agents are taken to include both living organisms and toxins.
2. The focus group did not consider classical microbiology except to provide background for the sake of comparison.
3. The focus group did not consider biological weapons that may be used against animals, plants, or inanimate objects.

4. Zilinskas, RA. 1986. "Recombinant DNA Research and Biological Warfare," in RA. Zilinskas & B. K. Zimmerman, *The Gene Splicing Wars. Reflections on the Recombinant DNA Controversy*, (New York: Macmillan Publishers), pp. 167-203.
5. Harrison, R. L. and B. C. Bonning, 2000. "Use of scorpion neurotoxins to improve the insecticidal activity of *Rachiplusia ou multicapsid*" *Biological Control* 17 (2): 191-201.
6. Currently, indications are that intragroup genetic variability is greater than genetic variability between groups. Nevertheless, information generated by the HGP is likely to eventually identify specific genetic differences between populations.
7. Division of Microbiology and infectious Diseases, National Institute of Allergy and Infectious Diseases, 2000. *The Jordan Report 2000: Accelerated Development of Vaccines* (Washington, DC., National Institute of Allergy and Infectious Disease).
8. PCR is a method for rapidly amplifying a small amount of genetic material to such an extent it can be easily identified.
9. Some investigators now believe that the human genome contains more genes than previously thought, perhaps as many as 140,000.
10. Hieter, Philip and Mark Boguski, 1997. "*Functional genomics: it's all how you read it*" *Science* 278:601-602.
11. Some claim that Soviet scientists combined smallpox and Ebola viruses (see Preston, Richard, 1998. "The bioweaponers;" *New Yorker*, March 9, pp. 52-65.) This probably did not happen. However, the techniques used for the genetic manipulation of the vaccinia virus would not differ from those that would be used to genetically manipulate the smallpox virus.
12. Del Giudice, G. and R. Rappuoli, 1999. "*Genetically derived toxoids for use as vaccines and adjuvants*" *Vaccine* 17: S44-S52