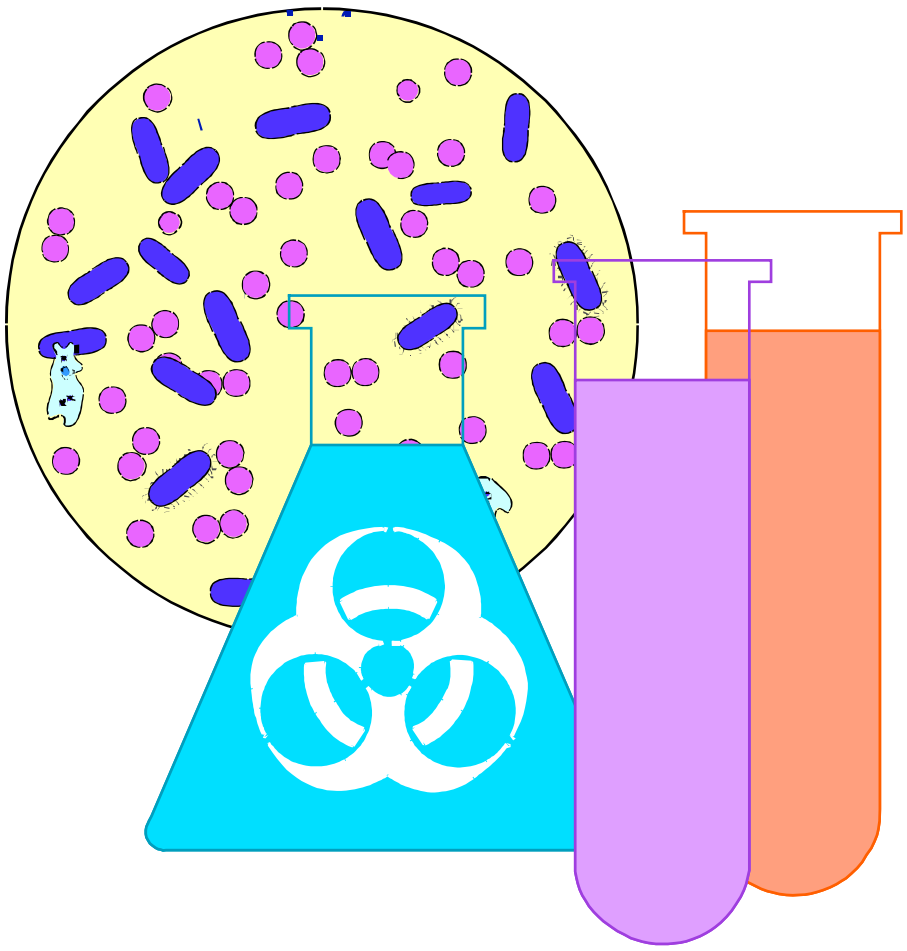
ADVANCES IN BIOTECHNOLOGY AND GENETIC ENGINEERING:

Implications for the Development of New Biological Warfare Agents



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Prepared by the Office of the Deputy Assistant to the Secretary of
Defense for Chemical and Biological Defense
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EXECUTIVE SUMMARY

The conferees on the FY96 National Defense Authorization Conference noted with concern that the recent progress in biotechnology could potentially lead to the development of new biological warfare (BW) agents and capabilities among potential adversaries of the United States. This report provides information to the Congressional defense committees on:

- the national security threats posed by such potential developments of new agents through advances in biotechnology and genetic engineering;
- recommendations related to reducing the impact of progress in these areas;
- the utility of increased emphasis on research and development of medical countermeasures related to mid-term or far-term biowarfare threat agents; and
- other measures that could reduce the threat of these technological advances and reduce the threat of biological agent and weapons proliferation.

Acquisition of biotechnology and biological weapons' capabilities is considerably easier than was the case in the 1940s and 1950s. There has been an explosion in biotechnologies and genetic engineering technologies—all of which have legitimate civilian applications—which may enable proliferation. As Gordon Oehler, Director of DCI's Non-Proliferation Center, testified before the Senate Armed Services Committee, March 27, 1996, "we see a continuing pursuit by many countries to acquire chemical and biological weapons. The chilling reality is that these materials and technologies are more accessible now than at any other time in history."

This report focuses on these issues and provides the basis for more detailed discussion of funding and program priorities, particularly in the area of medical biological defense research.

Despite revolutionary developments in biotechnology, great costs and technological barriers still block the ready development of novel BW agents. The detailed understanding of genetic structures has not yet led to the ability to control these genetic mechanisms. One can be certain, however, that significant advances in biotechnology will continue. It is viewed that classical BW threat agents pose the greatest concerns for the near- and mid-term. Far-term threats are not so easily predicted. Biotechnology is a two-edged sword. While providing an increasing number of methods for the protection of U.S. forces, biotechnology also sheds new light on methods to kill or incapacitate with ferocity.

Investment in medical science and technology base (S&T) programs has a high payoff in providing products that support readiness and battle sustainment for small costs relative to the overall DoD S&T budget. The fiscal S&T guidance funding profile currently is adequate only to address the highest threat priorities, and to sustain "core" capabilities needed to prepare to respond to new high priority scenarios (*e.g.*, counterterrorism). Resources are not entirely adequate to cope with lower priority items, including long-term threats from novel BW agents. Continued and stable investment will ensure that the Department's core S&T capability will be able to adapt to evolving threats.

RECOMMENDATIONS

- Provide funding of new basic research and scientific investigations of biotechnology, genetic engineering, and other areas with potential applications for biological warfare defense products, *i.e.*, monoclonal antibodies, genetically engineered vaccines and drugs.

- Determine the impact of personnel and resource reductions to DoD Medical Chemical and Biological Research Laboratories, especially focusing on the ability of the Department to maintain its core science and technology base capabilities in these areas.

- Ensure the appropriate levels of funding for unfunded requirements and program requirements unique to biological defense (for example, Food and Drug Administration licensure of medical products).

- Continue educating senior leaders on the nature of the threat and possible approaches to defense.

- Continue to exploit the very strong US commercial/university activity in biology and biotechnology; develop a Biotechnology Advisory Council with senior industry/university representation, working with ATSD(NCB) and reporting to USD(A&T) to bring the latest technologies and advances to rapid fruition.

- Intelligence efforts must emphasize collection and analysis of nations' "dual-use" biological industrial and scientific capabilities and develop indications and warning of adversarial use of these dual-use capabilities.

- Increase training for medical personnel for biological and chemical warfare casualty management.

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The conferees on the FY96 National Defense Authorization Conference Report (House Report 104-450, p.730) noted with concern that the recent progress in biotechnology could potentially lead to the development of new biological warfare (BW) agents and capabilities among potential adversaries of the United States. The Department of Defense (DoD) was directed to report to the congressional defense committees on:

- the national security threats posed by such potential developments of new agents through advances in biotechnology and genetic engineering;
- recommendations related to reducing the impact of progress in these areas;
- the utility of increased emphasis on research and development of medical countermeasures related to mid-term or far-term biowarfare threat agents; and
- other measures that could reduce the threat of these technological advances and reduce the threat of biological agent and weapons proliferation.

This report will address each of these issues and provide the basis for more detailed discussion of funding and program priorities, particularly in the area of medical biological defense research.

1.0 THE BIOLOGICAL WARFARE THREAT

Biological weapons are the most problematic of the weapons of mass destruction (WMD). They have the greatest potential for damage of any weapon. They are accessible to all countries, with few barriers to developing them with a modest level of effort. The current level of sophistication of BW is comparatively low, but there is enormous potential—based on advances in modern molecular biology, fermentation and drug delivery technology—for making sophisticated weapons. It is important for the United States to respond to the proliferation of these WMD. There remains a tendency to say biological weapons are "too hard to deal with." A vigorous and productive defensive program is possible and will do much to mitigate the risk to the United States and it allies.

Biotechnology will lead to potentially new BW agents and capabilities. The ability to modify microbial agents existed even before the 1970s, when revolutionary new genetic engineering techniques began to be introduced, but the enterprise tended to be slow and unpredictable. (Annex A highlights some of the key developments in biotechnology over the past few decades that have created the "biotechnology revolution.") With today's much more powerful techniques, infectious organisms can be modified to bring about disease in different ways and to enable relatively benign organisms to cause harmful effects. Genetic engineering gives the BW developer a powerful tool with which to pursue agents that defeat the protective and treatment protocols of the prospective adversary. Genetically engineered micro-organisms also "raise the technology hurdle" that must be overcome to provide for effective detection, identification, and early warning of BW attacks.

The future likelihood of infectious agents being created for BW purposes will be influenced by several *technological trends,* of which four of the most significant are:

1) genetically engineered "vectors" in the form of modified infectious organisms will be increasingly employed as therapeutic tools in medicine, and the techniques will become more widely available;

2) strides will be made in the understanding of infectious disease mechanisms and in microbial genetics that are responsible for disease processes;

3) an increased understanding of the human immune system function and other disease mechanisms will in turn shed light on the circumstances that cause individual susceptibility to infectious disease; and

4) Vaccines and antidotes will be improved over the far-term, perhaps to the point where "classical" BW agents will offer less utility as a means of causing casualties.

The question of what disease-causing organisms might replace those that are currently available is critical to understanding the future threat from BW agents. The Soviet example is instructive. Despite the efforts of a major industrial power, current BW agents do not represent a significant or even incremental improvement over what was available decades ago. This fact suggests that nations with current programs, and especially new entrants, will find the "classic" BW agents difficult to improve. Nevertheless, one recurring theme of BW threat forecasts is the expected appearance of "new" disease organism threats.

In a 1992 report on emerging infectious diseases, The Institute of Medicine found that "Pathogenic microbes can be resilient, dangerous foes. Although it is impossible to predict their individual emergence in time and place, we can be confident that new microbial diseases will emerge." Thus, the emergence of new BW agents as a result of biotechnology and genetic engineering may be complemented by natural selection. Examples of recent new pathogens (though not necessarily ideal BW agents) include (1) the human immunodeficiency virus (HIV), the causative agent of AIDS, and (2) *Streptococcus pneumoniae* S23F, a recently discovered naturally-occurring strain of pneumonia resistant to at least six of the more commonly used antibiotics.

The classical BW threat agents (see table 1) pose the greatest concern for the near- and mid-term. Far-term threats are not so easily predicted. Despite revolutionary developments in biotechnology, great costs and technological barriers still block the ready development of novel BW agents. The detailed understanding of genetic structures has not yet led to the ability to control these genetic mechanisms. (For example, scientists were able to clone and sequence the entire HIV genome in 1984. However, despite tremendous efforts, an effective vaccine has not yet been developed.) One can be certain, however, that significant advances in biotechnology will continue.

Table 1. BW Threat Agents

- Anthrax
- Bioregulators
- Botulinal toxins
- Brucellosis
- Cholera
- Clostridium perfringens
- Encephalomyelitis viruses
- Glanders
- Hemorrhagic Fever viruses
- Mycotoxins
- Neurotoxins
- Staphylococcal Enterotoxin B (SEB)
- Plague
- Q-fever
- Ricin
- Shigella
- Smallpox
- Tularemia
- Typhus

1.1 Characteristics of Biological Agents

Certain characteristics are *required* for an organism or substance to be an effective biological agent. Additional characteristics that will enhance their value under varied conditions of use are *desired*. The selection of a particular biological agent will be governed not only by the effect desired but also by the agent's characteristics and its ability to withstand environmental conditions. All these conditions cannot usually be fulfilled by any one agent; therefore, in making a selection, some compromise may have to be made between characteristics ranging from optimal to minimal desirability. Table 2 shows characteristics of biological agent that were considered by the U.S. military when planning to employ BW agents prior to President Nixon's ban on the use or possession of BW agents in 1969.

Table 2. Characteristics of Biological Agents

Requirements.

(1) Consistently produce a given effect (death, disability or plant damage).
(2) Be manufacturable on a large scale.
(3) Be stable under production and storage condition, in munitions, and during transportation.
(4) Be capable of efficient dissemination.
(5) Be stable after dissemination.

Desirable characteristics:

(1) Possible for the using forces to protect against.
(2) Difficult for a potential enemy to detect or protect against.
(3) A short and predictable incubation period.
(4) A short and predictable persistency if the contaminated area is to be promptly occupied by friendly troops.
(5) Capable of: (a) infecting more than one kind of target (for example, man and animals) through more than one portal of entry. (b) Being disseminated by various means. (c) Producing desired psychological effects.

Source: Adapted from U.S. Departments of Army and Air Force. *Military biology and biological agents*. Departments of Army and Air Force manual TM 3-216/AFM 355-56. 12 March 1964.

Additional characteristics that might be considered by a BW agent developer include *deniability* and *control or manipulation of symptoms*. Deniability would be a desirable characteristic if a country seeks to maintain the appearance of compliance with the Biological and Toxin Weapons Convention. This may result in the selection of a BW agent that results in a

disease endemic to a region. Investigations of an unusual outbreak of an infectious disease may be inconclusive. Even if there were strong indications that the disease were the result of a BW attack, the lack of conclusive evidence may result in no response to the attack.

The control and manipulation of symptoms may also be desirable. The purpose of manipulating symptoms would be to confuse and delay the diagnosis and treatment of the disease, potentially resulting in increased casualties. A new strain of a classical BW agent could result in different or multiple symptoms that normally would not be expected. For example, a new strain may result in a rash, high fever, or have a longer than expected duration.

1.2 The Potential Impact of Biotechnology and Genetic Engineering

The revolution in biotechnology facilitates an evolution in the BW threat. This revolution is the third major technological "wave" in the history of chemical and biological warfare developments. Each wave has resulted from advances in legitimate scientific advances. The *first wave* began with World War I and included the employment of commercial chemical compounds (*e.g.*, chlorine, phosgene) as warfare agents. Modifications led to the development of mustard agents during this period. The *second wave* began in the 1940s with the creation of the first cholinesterase inhibitor—the nerve agent tabun (GA). This wave continued through the 1960s, and has not clearly ended. It has resulted in the development of powerful cholinesterase inhibitors, including sarin (GB), VX, and most recently the Novichok family of agents. The third wave began in the 1970s with the biotechnology revolution. Scientific and technological advances have facilitated the development of genetically engineered agents. Biotechnology has also led to a blurring of the distinction between chemical and biological agents with the advent of the "mid-spectrum" agents. Mid-spectrum agents have characteristics of chemical and biological agents and include such compounds as toxins, bioregulators, and physiologically active compounds (PACs).

The extreme lethality of BW agents has long been known. Combined with the gruesome symptoms caused by some biological agents, biological warfare has frightening and potentially devastating potential. The most lethal biological agents can be hundreds to thousands of times more lethal per unit than the most lethal chemical warfare agents. Table 3 illustrates the comparative theoretical lethality of a chemical agent (VX) and biological agent (Botulinum toxin) based on materiel declared by Iraq following DESERT STORM.

Table 3. Comparative Lethality of Botulinum Toxin and VX

	Botulinum Toxin	VX
Lethal Dose (LD_{50})/70 kg	0.14 micrograms (μg)	20 milligrams = 20,000 μg
Quantity in Iraqi Stockpile	11,800 liters	500 tons†
Theoretical Lethal Doses*	86×10^{12} (trillion)	23×10^{9} (billion)

*by injection

†approximately 500,000 liters

However, lethality is only one of many characteristics necessary to consider in the development, production, and employment of a BW agent. Figure 1 illustrates the numerous characteristics that need to be controlled for a highly effective BW agent. Historically, the

accentuation of one characteristic often resulted in the attenuation of one or more other characteristics, possibly even rendering the modified agent ineffective as a weapon. Advances in biotechnology, genetic engineering, and related scientific fields provide increasing potential to control more of these factors, possibly leading to the ability to use BW agents as tactical battlefield weapons.

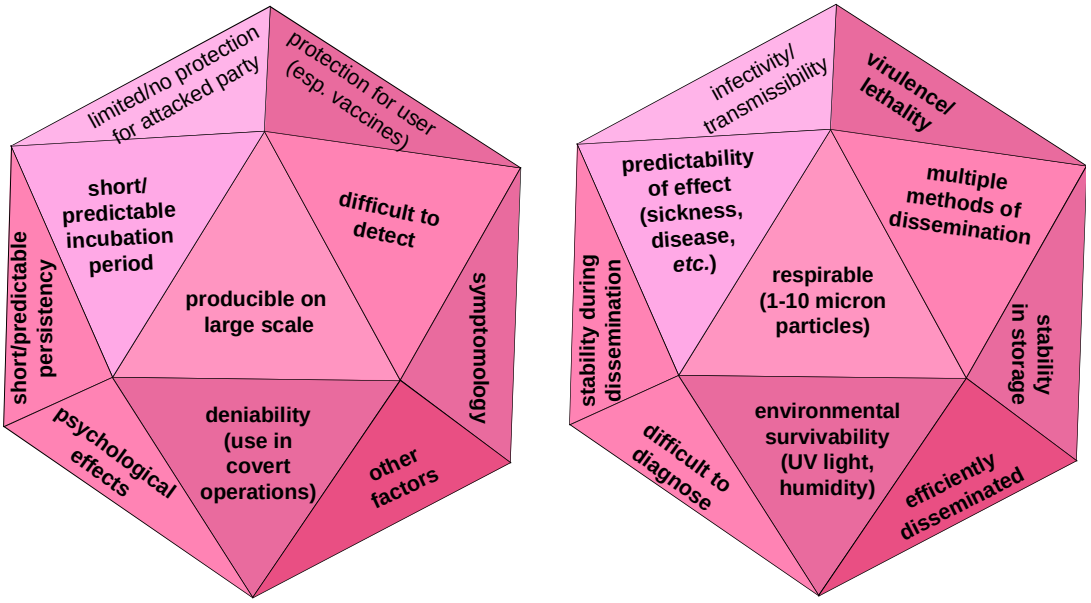

Figure 1. Balancing Characteristics of a BW Agent

Biotechnology is a two-edged sword. While providing an increasing number of methods for the protection of U.S. forces, biotechnology also sheds new light on methods to kill or incapacitate with unprecedented ferocity.

The ***potential types of novel biological agents*** (microorganisms) that could be produced through genetic engineering methodologies are:

1) Benign microorganisms, genetically altered to produce a toxin, venom subfraction, or endogenous bioregulator.

2) Microorganisms resistant to antibiotics, standard vaccines and therapeutics.

3) Microorganisms with enhanced aerosol and environmental stability.

4) Immunologically altered microorganisms able to defeat standard identification, detection, and diagnostic methods.

5) Combinations of one through four with improved delivery systems.

It is noteworthy that each of these techniques recognizes the extreme lethality of BW agents and tries to exploit this potential by developing methods to efficiently deliver and control the agents on the battlefield.

With the advent of various genetic engineering techniques, biological compounds such as human insulin, growth hormone, and blood clotting factors can be produced in fermentors containing cultures of microorganisms altered to include the genes that code for the elaboration of these proteins. It is this technology that potentially affords a country with a competent university system, access to a pharmaceutical industry, and the political/military will to pursue a BW program the potential ability to create infectious organisms with novel properties.

Another example of genetic engineering that may improve the ability to use biological weapons would be to enhance the ability of BW agents to survive under normally hostile environmental conditions. This may be done by splicing a gene from a toxin or other lethal agent with an otherwise non-lethal spore forming bacteria. The bacteria's spore will provide increased protection against degradation from ultraviolet (UV) light (that is, sunlight), humidity, heat, or other environmental factors. Another technique may be to microencapsulate a toxin or virus so that it is protected from harsh environmental factors. The encapsulating wall can be designed so that it is a respirable size (approximately 1 to 10 microns), will survive harsh environmental conditions, yet degrade to release the pathogen after being inhaled. Protection against harsh environmental factors could allow a potential aggressor to employ BW agents in what would be otherwise poor conditions. For example, a pathogen may decay quickly in sunlight. As shown in Table 4, if a technique could be used to improve a pathogen's rate of decay from 5% per minute to 0.5% per minute, it could survive for more than two hours over a target area (rather than a few minutes) exposing a greater number of personnel and increasing the probability that it will have effect on those in the target area.

Table 4. Aerobiological Decay

Rate of Decay (%/min)	**Half-Life** (min)*
0.5	138
1	69
2	34
5	13
10	6

*rounded to nearest minute

Another possible approach is to employ viruses that have been modified so that they do not result in the customary symptoms such as fever or malaise, but some other far more debilitating effect. By such alteration, the cellular machinery of the host body can be used for producing an incapacitating or lethal substance. A notional example is the use of a benign virus, such as vaccinia, as a "vector" for the genetic instructions for elaboration of a toxic compound (*e.g.*, cobra toxin) within the cells of the host. The vaccinia virus currently is being used in developing new means of immunization against other infectious organisms. Using existing technologies, researchers can splice into the vector virus genetic instructions to produce a toxin or some other factor, such as "bioregulators" with harmful physiological or psychological

properties. This approach, which offers a means for producing and delivering a detrimental substance from within the body over an extended time, would make diagnosis and treatment very difficult. In addition to virus vectors, modified bacteria, rickettsia, and fungi also could be used to bring about infectious conditions with novel effects.

Ongoing scientific research into the functioning of disease organisms also is expected to provide insights for the development of advanced medical defenses against new and emerging BW threats. Current examples of infectious organisms that are attracting particular attention are human immunodeficiency virus (HIV), the causative agent of AIDS), hantaviruses (hemorrhagic fever causing agents, such as Ebola), and the “flesh-eating” streptococcus bacteria. The streptococcus example is illustrative. While not a “new” medical problem, the particular strain involved is capable of producing a combination of toxins that results in simultaneous toxic shock and rapid spread of tissue breakdown. Once it is well established, the infection is very difficult to control with antibiotics. Although the “natural” form of this organism may not have significant potential as an aerosol threat agent, those seeking new infectious agents for military use could investigate its mechanisms of action.

2.0 COUNTERING THE BW THREAT

"We continue to maximize our technological advantage over any potential foe, to give us dominance on any battlefield in the world.... [I]f we cannot prevent or deter conflict we will be able to defeat an aggressor quickly with a minimum of casualties."

– Secretary of Defense Perry, May 13, 1996

One of the tenets of the Defense Science and Technology Strategy is the prevention of technological surprise. Technological surprise historically occurs when new technology is employed with a surprising concept of operations. This requires good intelligence on capabilities and intentions of potential adversaries. It also requires that the U.S. science and technology community maintain a continuing awareness, through its own scientific investigation, of emerging technology that could have military applications. Defense scientists and engineers must be poised to react rapidly to an innovative use of technology by potential adversaries. Advanced Technology Demonstrations will speed consideration of alternative operational concepts for U.S. employment of new technology.

To counter potentially new and more effective BW agents, a broad array of counter-measures is available. The following sections of this report examine medical countermeasures and other countermeasures, including preventive measures, deterrence, intelligence, detection and identification technologies, non-medical protective measures, decontamination measures, and other measures such as counterterrorism. Medical countermeasures are critical to an effective biological defense program. All other measures are aimed at preventing the use, effective dissemination, or contact with biological agents. Only medical countermeasures provide protection to an individual once he or she has been exposed to a BW agent.

2.1 UTILITY OF MEDICAL COUNTERMEASURES

The strategy for the medical support capabilities to deter and counteract BW use against U.S. Forces was developed to:

- address the most probable threats;
- field capabilities for two Major Regional Contingencies (MRCs);
- provide medical products necessary to allow personnel to operate and sustain operations on a BW agent contaminated battlefield;
- complete critical acquisition of medical support materiel;
- consolidate requirements for medical countermeasures across Services; and,
- provide a responsive medical modernization strategy to prevent or treat BW casualties to maximize protection and return to duty, respectively.

Drugs, vaccines, medical devices, and therapeutics are being developed to prevent, diagnose, and treat casualties. All products developed under this program require full Food and Drug Administration (FDA) approval and licensure. Science and Technology Base initiatives continue to focus on specific vaccines, drugs and devices. Current capabilities include an anthrax

vaccine, an array of antibiotics (including tetracycline and ciproflaxin), and training in biological weapons casualty management.

Medical Biological Defense: Responding to Mid- and Far-term Biowarfare Threats

Advanced development and fielding (FDA approval) during the mid-term is anticipated for several vaccines, including the following:

- Multivalent botulinum vaccine,
- Tularemia vaccine,
- Q-Fever vaccine,
- Venezuelan equine encephalomyelitis recombinant vaccine,
- Improved Plague vaccine,
- Ricin vaccine,
- Smallpox vaccine, and
- Staphylococcus enterotoxin B vaccine.

Additionally, rapid, forward-deployable medical diagnostic tests for early screening of patients are being developed for deployment in the mid-term.

Far-term enhancements include confirmatory medical diagnostics. Long-range strategies focus on providing countermeasures for novel, or bio-engineered biological threat agents along with appropriate diagnostic methods. Strategies using recombinant technologies or naked DNA will be emphasized to develop highly tailored vaccines.

Investment in medical biological defense science and technology base (S&T) programs is essential to provide the countermeasures necessary to protect and treat operating forces on the BW agent contaminated battlefield. The Medical Biological Defense Research Program (MBDRP) is fully responsive to joint warfighting needs and priorities. The S&T investment yields vaccines, drugs, field medical devices, field diagnostic kits, and patient management procedures. Continued investment is fundamental to the development and fielding of medical solutions to sustain, prevent, diagnose and treat service members engaged in any operation with the potential need for defensive measures against biological weapons. Investment in medical S&T has a high payoff in providing products that support readiness and battle sustainment for small costs relative to the overall DoD S&T budget.

The fiscal S&T guidance funding profile currently is adequate only to address the highest threat priorities, and to sustain "core" capabilities needed to respond to any new conflict scenarios (*e.g.*, counterterrorism). Funding is not adequate to completely meet all the current high-priority product timelines to produce the final-stage prototype human-use vaccines (Good Manufacturing Practice (GMP) level production) and Good Laboratory Practice (GLP) studies needed for Food and Drug Administration (FDA) data submission packages. Emerging validated threat agents are not adequately addressed with the programmed resources. Furthermore, any additional reduction in funding resources from fiscal guidance baseline will substantially delay program milestones, will compromise the development of countermeasures, and, if tied to

personnel, will compromise the Department's core S&T capability. Additional funding is required to acquire needed GMP produced biologics for advanced development of vaccines and medical diagnostic reagents. These GMP produced materials are highly purified and characterized biologics (*e.g.*, final vaccine preparations which are required to initiate human use safety trials for eventual FDA licensure.

Other medical biological defense S&T programs seeking funds are to develop medical tests to rapidly diagnose smallpox from human clinical specimens following agent exposure under battlefield conditions and to develop anti-viral drugs against smallpox. Current capabilities do not permit rapid, definitive analysis of the virus in clinical specimens. No anti-viral drugs exist to treat post-exposure casualties which would be expected from use of this BW threat agent against unwarned, unprotected personnel.

Reference Laboratory for Critical National Response Requirements: The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) has existing capabilities which can evaluate terrorist incidents from the initial communication of the threat or incident to its resolution. These capabilities include: technical expertise to assist in the evaluation of threat capability in relation to specific biological agent or agents, assist in the evaluation of delivery methods and their medical impacts, identification of biological agents in samples (medical and environmental), technical and biomedical expertise required to protect personnel responding to such a terrorist incident or to decontaminate personnel and facilities, technical expertise to accomplish medical and operational planning, special vaccines for personnel who respond to such incidents, and specialized transport of limited numbers of biological casualties under containment conditions to a receiving medical care facility. Additional funding is required to maintain USAMRIID's special containment facilities and a small core of personnel, equipment and validated reagents as a one-of-a-kind national resource capable at a moments notice to respond to: (1) domestic biological threats, (2) overseas biological threats, and (3) special munitions incidents in CONUS. Additional funding, would be utilized to maintain the tech base capability for contingency operations as a national, confirmatory reference laboratory on a long-term basis. This support is required to maintain and keep up-to-date USAMRIID's unique capabilities in an operational readiness posture and to address unique and new issues for counterterrorism initiatives that are currently unfunded. It is important that these capabilities complement and continue close coordination with operational capabilities of the Services and the Commanders-in-Chief (CINCs) which respond to BW threats.

Medical Biological Defense Training

In the near- through mid-term, DoD is seeking to enhance *Medical Biological Response Training*. This enhancement provides trained and equipped medical personnel to respond to biological attacks on US Forces. The medical biological assessment response team will be capable of collecting and transporting biomedical samples from patients and deceased to CONUS as well as initiating an epidemiological assessment. Central funding will provide focused support and training of the response team.

Field Hospital Training. Provides central funding and management of medical NBC defense training. Funding will be used to conduct enhanced NBC medical training and training plans. The funding will increase training opportunities and improve unit medical NBC training.

NBC Joint Medical Evaluation. Conduct a field tested, Joint Service defined medical NBC education and evaluation program. The program will lead the student through a reproducible, high quality NBC education and training program based on adaptive self-paced cognitive reactive model. The program provides measurement and retraining through feedback medical algorithms.

NBC Medical Training. Activates the AMEDD Center and School as a Primary Distance Learning Facility for Medical NBC which includes the procurement of equipment, manpower resources and development and conversion of existing instructional material, and installation of training technology infrastructure to support distance learning. This will expand the AMEDD Center and School medical NBC educational program and be compatible with the existing Navy medical education system.

AMEDD Medical NBC Training Courses. Establishes enhanced medical NBC training programs through the AMEDD Center and School. The comprehensive medical NBC courses would include NBC Medical Specialty training, NBC Defense Medical Unit Training Simulations, Upgrade to NBC Defense Medical Training facilities, Medical NBC Defense Distributed Training, NBC Physician Basic Course Training, NBC Medical Doctrine Improvement, Medical Chemical and Biological Casualties (MCBC) Training Course.

Medical Biological Defense Procurement

Procurement of medical biological defense products focuses on biological defense vaccines and medical diagnostic devices. DoD Guidance assigns highest priority on developing capability for production of vaccines for biological agent defense. A new contract will likely be established for biological defense vaccine advanced development, licensure, production and stockpiling capability by the 2nd Quarter FY 1997. This contract will provide the vehicle for developing and acquiring new vaccines. The botulinum multivalent vaccine, used during the Gulf War, remains in a relatively early phase of the investigational new drug (IND) process, and complete technical data packages for this vaccine (and most others under development) are not available. To apply for FDA licensure and initiate production, considerable product safety and efficacy data along with manufacturing data are required for these new vaccines. An independent cost estimate shows that significant funding must be made available to complete all developmental work, including any facilities improvement or renovation, prior to FDA licensing. Increased research, development, test and evaluation (RDT&E) costs reflect the unique FDA requirement for biological products that (a) the manufacturing establishment and the product be licensed, and (b) that data on “production prove out lots” from the operational manufacturing facility be submitted to the FDA as part of the license application. To meet the unique FDA regulatory requirements for licensing these products in the near-term, procurement funding must be reallocated to RDT&E to complete advanced development, obtain licensure, and initiate production as quickly as possible.

2.2 UTILITY OF OTHER COUNTERMEASURES

The Department of Defense (DoD) is currently providing this leadership through a three-part strategy: 1) reduce the threat, by leading the U.S. effort to help the former Soviet Union republics reduce, dismantle, safeguard, and even eliminate WMD; 2) deter against the threat, by maintaining strong conventional forces and a smaller but robust nuclear deterrent force; 3) defend against the threat through the Defense Counterproliferation Initiative.

The counterproliferation initiative involves a wide range of activities that help to prevent, protect against, and even reverse the danger from spreading WMD technology and missiles that can deliver them. These efforts include developing systems that can intercept or destroy these weapons, providing vaccines and protective suits for our troops, keeping track of the movement of weapons and technology, and providing unique DoD support for various nonproliferation agreements.

Controlling or containing proliferation involving terrorist groups is particularly difficult because these groups evade or defy recognized export controls or nonproliferation regimes. Should these groups acquire WMD, they may be more inclined to employ them in order to achieve their goals than would a member in good standing of the international community.

The 1996 *Report on Activities and Programs for Countering Proliferation*, DoD outlines a multi-tiered response to countering WMD. Considering the complexities of facing an adversary armed with WMD, proliferation prevention activities are given a high priority. Realizing, however, that efforts to halt the proliferation of WMD and their means of delivery have not been entirely successful, DoD must prepare U.S. armed forces to fight, survive and prevail in any conflict involving the use of these weapons by an adversary. In addition to the medical countermeasures described above, the following capability areas are being pursued to counter biological warfare: (1) proliferation prevention, (2) strategic and tactical intelligence, (3) battlefield surveillance, (4) counterforce, (5) active defenses, (6) passive defenses, and (7) countering paramilitary/covert and terrorist WMD threats.

Proliferation Prevention

Proliferation prevention is defined as efforts to deny attempts by would-be proliferants to acquire or expand their WMD capabilities by: providing inspection, verification and enforcement support for nonproliferation treaties and WMD control regimes; supporting export control activities; assisting in the identification of potential proliferants before they can acquire or expand their WMD capabilities; and, if so directed by the National Command Authority (NCA), planning and conducting interdiction missions.

The way we reduce the risk from weapons of mass destruction has changed dramatically from the days of the Cold War. The simple threat of retaliation that worked during the Cold War is not necessarily enough to deter terrorists or aggressive regimes from using WMD.

Programs such as the Nunn-Lugar Cooperative Threat Reduction program, which is hastening the dismantlement of Russia' nuclear weapon systems, and the Nuclear Non-Proliferation Treaty, which will serve to stem regional or even new global arms races are prime examples of what is needed. These successes demonstrate that the U.S. diplomatic leadership in the world is critical to nonproliferation of nuclear, biological, and chemical weapons.

The Defense Counterproliferation Initiative places great emphasis on international cooperation in preparation for future crises or conflicts where the threat or use of NBC weapons may be present. DoD is currently beginning other cooperative efforts with allies. A defense science symposium involving participants from the United States, United Kingdom, Canada, and Australia was conducted in the United States in March 1995. This symposium focused on counterproliferation technology applications and on the identification of opportunities for collaborative research and development to enhance counterproliferation capabilities. The United States, Canada. and the United Kingdom, have created a cooperative R&D program to improve capabilities for detecting, characterizing, and providing protection against biological and chemical agents based on lessons learned during the Gulf War. International norms and standards make an important contribution to proliferation prevention. In addition to creating an atmosphere of restraint, they provide the preconditions, *e.g.*, inspections, that impede proliferation. These international norms can be specifically agreed to in export control and arms control agreements or they can result from informal arrangements between states. An example of a great success in the area of norm establishment has been DoD support for the unconditional and indefinite extension of the Non-Proliferation Treaty (NPT).

Strategic and Tactical Intelligence

Strategic and tactical intelligence to support counterproliferation is defined as efforts to provide to policy and operational organizations actionable foreign intelligence on the identity and characterization of activities of existing or emerging proliferant states and groups, in order to support U.S. efforts to prevent the acquisition of weapons and technology, cap or roll back existing programs, deter weapons use, and adapt military forces and emergency assets to respond to threats.

Intelligence and international cooperation are the critical areas to counter the terrorist threat. The Intelligence Community must provide accurate and timely intelligence assessments on the motivations and clandestine procurement networks use by such elements. This is a demanding set of requirements. The dual-use nature of many technologies involved in WMD and delivery systems development complicate these tasks. The Defense Intelligence Agency (DIA) remains the prime conduit for national-level intelligence support to the Defense Department. To better focus its intelligence support to counterproliferation, it created an Office for Counterproliferation and Nuclear, Biological, and Chemical Assessments.

Battlefield Surveillance

Battlefield surveillance to support counterproliferation is defined as efforts to detect, identify and characterize WMD forces and associated elements (using DoD and intelligence

assets) in a timely manner to support combat operations, such as targeting and mission/strike planning activities, and provide timely post-attack and battle damage assessment (BDA). In the case of biological weapons, programs are characterized by a variety of detection, identification, and warning capabilities described under passive defense.

Counterforce

Counterforce to support counterproliferation is defined as efforts to target, plan attacks, deny, interdict or destroy, and rapidly plan restrikes as necessary against adversarial WMD forces and their supporting infrastructure elements while minimizing collateral effects.

Most counterforce programs are designed to counter many types of threats, including WMD. One key programs includes several closely related efforts to develop new warheads capable of accurately destroying a variety of hardened and deeply buried targets. The key counterforce program designed to counter biological weapons is the agent defeat/agent neutralization warhead. This capability may offer in the mid-term a capability for the *in situ* destruction of biological agents within munitions or storage containers without releasing an active biological agents into the atmosphere.

Active Defense

Active defense is defined as efforts to protect U.S., allied and coalition forces and noncombatants by intercepting and destroying or neutralizing NBC warheads delivered by ballistic and cruise missiles, while minimizing collateral effects that might arise during all phases of intercept.

Several programs are being developed by the Services and the Ballistic Missile Defense Organization (BMDO) to counter a variety of threats posed by ballistic and cruise missiles. Strong support and stable funding levels offer a critical capability to counter the greatest threat for the long-range delivery of weapons of mass destruction. Russia and China already have developed missiles capable of reaching the continental United States. It is believed that early in the next century, North Korea may deploy a missile capable of striking portions of the United States. The missile threat from North Korea is compounded by its extreme economic problems and its demonstrated willingness to sell weapons technologies for hard currency.

Passive Defense

Passive defense is defined as efforts to protect U.S., allied, and coalition forces against NBC effects associated with WMD use, including: measures to detect and identify NBC agents, individual and collective protection equipment for combat use, NBC medical response, and NBC decontamination technologies.

Within passive defense, biological defense is developed around a ***system-of-systems architecture***. The research, development, and acquisition of non-medical and medical biodefense capabilities is supported by five capability areas: (1) contamination avoidance, (2) individual

protection, (3) collective protection, (4) decontamination, and (5) medical programs. All capability areas are interrelated and critical to the defense of our forces.

In addition to medical initiatives described in section 2.1, contamination avoidance is the highest priority for countering biological weapons. DoD has recently begun fielding of initial biological detection capabilities, including the Biological Integrated Detection System (BIDS), the Long-Range Biological Standoff Detection System (LRBSDS), and the Interim Biological Agent Detector (IBAD).

Many of the biological identification systems rely on antigen specific identification. One S&T initiative, a neuron-based biosensor, may be able to provide detection of any compound that would cause physiological damage regardless of the antigen structure of the compound. This system offers the potential for countering any attempts by a potential adversary to genetically alter the antigen structure of a BW agent to avoid detection by antibody-based detection systems. Other key biological detection focus on the generic detection of aerosols or particulates in the atmosphere that are not natural formations (for example, an aerosol cloud appearing from a line source.) Such generic detection schemes are critical to support an effective biological defense architecture since they focus on detecting the delivery of BW agent rather than on the BW agent itself. Thus, even new genetically-engineered BW agents will be detectable. Specific initiatives for biological detection are described below.

Over the mid- to long-term, DoD is pursuing several initiatives to counter biological weapons. These initiatives are defined as Defense Technology Objectives (DTOs). Some of the key DTOs include the following:

> **Integrated Biodetection Advanced Technology Demonstration (ATD):** The Integrated Biological Detection ATD will demonstrate point detection and remote early warning of BW agents using two state-of-the-art technologies: an automated DNA diagnostic technology and a biological aerosol particle counter. The ATD will focus on point biosensors that incorporate DNA technology to identify biological agents with the highest possible degree of specificity and sensitivity. A rapid real-time aerosol warning system using small, laser-based particle counters will also be demonstrated. Its purpose is to provide an early warning/alert of a threat biological aerosol cloud to high value fixed assets.
>
> **Biological Early Warning Advanced Concept Technology Demonstration (ACTD):** The objective of this ACTD is to develop, demonstrate and field stand-off and remotely-employable point detection capabilities which can detect BW agents. These detection capabilities will include alarms which will be integrated into warning and reporting networks to promptly warn all personnel who may be exposed to BW contamination. This ACTD will evaluate the use of a helicopter-mounted eye-safe laser which can detect particulate clouds (with respirable particles in the 1–10 micron range) at distances of 20 to 50 kilometers, depending on particle density. This system will not identify or characterize the particulate matter. To identify the particulate cloud, miniaturized and sensitive detectors are to be evaluated that can be remotely employed through air-drop, artillery, or mounted on unmanned aerial vehicles. Small, low-power air samplers must also be developed and evaluated for remote deployments and may be integrated with biodetection systems.

Airbase/Port Biodetection ACTD: This ACTD will develop, demonstrate, and field extensive BW agent detection, protection, and hazard assessment capability to a few select airbases and ports located in CINCs Areas-of-Responsibility (AORs). The approach would: (1) define the requirements of a major fixed-site facility in conjunction with the BW threat, (2) analyze the placement of sensors, communications network, protection, and decontamination needs, (3) adapt operational concepts/procedures, and (4) define training and logistical support. Key components will be designed, fabricated, and demonstrated at a continental United States (CONUS) facility similar to outside CONUS (OCONUS) sites. This ACTD will also examine a capability to assemble and store a rapidly deployable capability.

Countering Paramilitary, Covert and Terrorist WMD Threats

Countering paramilitary, covert and terrorist WMD threats includes efforts to protect military and civilian personnel, facilities, and logistical/mobilization nodes from this special class of WMD threats both in the United States and abroad. The March 1995 nerve agent attack on the Tokyo subway revealed a vulnerability to attacks with chemical or biological weapons. The United States is not adequately prepared at this time to respond to a terrorist incident in the US involving WMD. However, many initiatives are underway to correct many of these shortfalls in the near-term.

DoD's peacetime responsibility to support Special Operations Forces and WMD antiterrorist operations was judged a high priority by the Secretary of Defense and the Joint Staff. Maintaining a high priority requires the continued support from Congress and the President.

Some key shortfalls being addressed include (1) examination of adding a mission and authority to DoD to conduct programs of assistance to Federal, state and local emergency preparedness personnel in the defense against possible terrorist use of chemical or biological agents; (2) resources for WMD training exercises which should include coordination with state and local agencies, testing capabilities of Federal, state, and local communities, more frequent full-field exercises, and better test of consequence management capabilities; and (3) examination of DoD resources for training of local and regional emergency preparedness personnel, on-call resources to support those personnel, and establishment and maintenance of assets deployable to events which might be the subject of terrorism and emergency response to terrorist events.

3.0 CONCLUSIONS

Since 1972 when the Biological Weapons Convention was signed, advances in biotechnology have greatly increased the capacity for virtually any country to develop a biological warfare capability. There has been an explosion of the technologies that enable BW proliferation, all of which have legitimate civilian applications and are inherently dual-use. As Gordon Oehler Director of DCI's Non-Proliferation Center, testified before the Senate Armed Services Committee, March 27, 1996, "we see a continuing pursuit by many countries to acquire chemical and biological weapons. The chilling reality is that these materials and technologies are more accessible now than at any other time in history." Despite revolutionary developments in biotechnology, great costs and technological barriers still block the ready development of novel BW agents. The detailed understanding of genetic structures has not yet led to the ability to control these genetic mechanisms. One can be certain, however, that significant advances in biotechnology will continue. It is viewed that classical BW threat agents pose the greatest concerns for the near- and mid-term. Far-term threats are not so easily predicted.

Institutions and programs are in place to support the counterproliferation and defensive efforts against an evolving BW threat. Continued support of the programs with additional manpower and resources where needed will result in a continued strong program and policy.

4.0 RECOMMENDATIONS

1) Provide funding of new basic research and scientific investigations of biotechnology, genetic engineering, and other areas with potential applications for biological warfare defense products, *i.e.*, monoclonal antibodies, genetically engineered vaccines and drugs.

2) Determine the impact of personnel and resource reductions to DoD Medical Chemical and Biological Research Laboratories, especially focusing on the ability of the Department to maintain its core science and technology base capabilities in these areas.

3) Ensure the appropriate levels of funding for unfunded requirements and program requirements unique to biological defense (for example, Food and Drug Administration licensure of medical products).

4) Continue educating senior leaders on the nature of the threat and possible approaches to defense.

5) Continue to exploit the very strong US commercial/university activity in biology and biotechnology; develop a Biotechnology Advisory Council with senior industry/university representation, working with ATSD(NCB) and reporting to USD(A&T) to bring the latest technologies and advances to rapid fruition.

6) Intelligence efforts must emphasize collection and analysis of nations' "dual-use" biological industrial and scientific capabilities and develop indications and warning of adversarial use of these dual-use capabilities.

7) Increase training for medical personnel for biological and chemical warfare casualty management.

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Annex A:
Highlights of the "Biotechnology Revolution": 1953–present*

1953 *Nature* magazine published James Watson's and Francis Crick's manuscript which described the double helix structure of DNA. The **discovery of the structure of DNA** resulted in an explosion of research in molecular biology and genetics, paving the way for the "biotechnology revolution."

1955 Seymour Benzer at Purdue University devised an experimental setup to map mutations within a short genetic region of a particular bacterial virus. Over a five-year period, Benzer mapped recombinations of genetic material that distinguished mutational changes that had taken place at adjacent base pairs.

1956 Heinz Fraenkel-Conrat took apart and reassembled the tobacco mosaic virus, demonstrating "self assembly."

1957 Francis Crick and George Gamov worked out the "**central dogma**," explaining how DNA functions to make protein. Their "sequence hypothesis" posited that the DNA sequence specifies the amino acid sequence in a protein. They also suggested that genetic information flows only in one direction, from DNA to messenger RNA to protein, the central concept of the central dogma.

1957 Matthew Meselson and Frank Stahl demonstrated the replication mechanism of DNA.

1958 Coenberg discovered and isolated DNA polymerase, which became the first enzyme used to make DNA in a test tube.

1958 The National Seed Storage Laboratory (NSSI) was opened in Fort Collins, Colorado, becoming the first long-term seed storage facility in the world.

1959 Francois Jacob and Jacques Monod established the existence of genetic regulation—mappable control functions located on the chromosome in the DNA sequence—which they named the repressor and operon. They also demonstrated the existence of proteins that have dual specificities.

1959 The steps in protein biosynthesis were delineated.

1959 Systemic fungicides were developed.

1961 Marshall Nirenberg built a strand of mRNA comprised only of the base uracil. This strand is called "poly-u," and by examining it Nirenberg discovered that UUU is the codon for phenylalanine. This was the first step in cracking the genetic code, which Nirenberg and colleagues succeeded in doing within five years.

1965 Scientists noticed that genes conveying antibiotic resistance in bacteria are often carried on small, supernumerary chromosomes called plasmids. This observation led to the classification of the plasmids.

1965 Harris and Watkins successfully fused mouse and human cells.

1966 The **genetic code was "cracked."** Marshall Nirenberg, Heinrich Mathaei, and Severo Ochoa demonstrated that a sequence of three nucleotide bases (a codon) determines each of 20 amino acids.

1967 Arthur Kornberg conducted a study using one strand of natural viral DNA to assemble 5,300 nucleotide building blocks. Kornberg's Stanford group then synthesized infectious viral DNA.

1967 Mary Weiss and Howard Green took a crucial step in human gene mapping with the publication of a technique for using human cells and mouse cells grown together in one culture. This was called **somatic-cell hybridization**.

1969 Leonard Herzenberg, a geneticist at Stanford, developed the fluorescence-activated cell sorter, which can identify up to 5,000 closely related animal cells.

1970 Peter Duesberg and Peter Vogt, virologists at UCSF, discovered the first oncogene in a virus. This SRC gene has since been implicated in many human cancers.

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* Adapted from "The Biotech Chronicles," *Access Excellence*, Genentech, Inc.,1996.

1970 Howard Temin and David Baltimore, working independently, first isolated "**reverse transcriptase**" a restriction enzyme that cuts DNA molecules at specific sites. Their work described how viral RNA that infects a host bacteria uses this enzyme to integrate its message into the host's DNA. This discovery allowed scientists to create clones and observe their function.

1970 Torbjorn Caspersson, L. Zech, and other colleagues in Sweden, published the first method for staining human or other mammalian chromosomes in such a way that banding patterns appear.

1972 The **Biological Weapons Convention** was signed. The purpose of this agreement was to prohibit the development, testing, and stockpiling of biological weapons. The treaty allows research for defensive purposes, such as to develop antidotes to biological weapons.

1972 Immunologist Hugh McDevitt, in an article in *Science*, reported observing genes that control immune responses to foreign substances. His observations suggested predictable, inherited susceptibility to some diseases.

1972 Paul Berg isolated and employed a restriction enzyme to cut DNA. Berg used ligase to paste two DNA strands together to form a hybrid circular molecule. This was **the first recombinant DNA molecule**.

1972 The first successful DNA cloning experiments were performed in California.

1972 In a letter to *Science*, Stanford biochemist Paul Berg and others called for the National Institutes of Health to enact guidelines for DNA splicing. Their letter recommended that scientists stop doing certain types of recombinant DNA experiments until questions of safety could be addressed. This letter was provoked by experiments planned by Berg, which had drawn vocal concern from the scientific community. Their concerns eventually led to the 1975 Asilomar Conference.

1973 Scientists for the first time successfully transferred deoxyribonucleic acid (DNA) from one life form into another. Stanley Cohen and Annie Chang of Stanford University and Herbert Boyer of UCSF "spliced" sections of viral DNA and bacterial DNA with the same restriction enzyme, creating a plasmid with dual antibiotic resistance. They then spliced this recombinant DNA molecule into the DNA of a bacteria, thereby producing **the first recombinant DNA organism**.

1973 Bruce Ames, a biochemist at UC Berkeley, developed a test to identify chemicals that damage DNA. The Ames Test becomes a widely used method to identify carcinogenic substances.

1973 The first human-gene mapping conference took place. The conference was inspired primarily by the rapid development in mapping by somatic-cell hybridization.

1974 The Proceedings of the National Academy of Sciences published a paper by Stanford geneticist Stanley Cohen and UCSF biochemist Herbert Boyer in which they demonstrated the expression of a foreign gene implanted in bacteria by recombinant DNA methods. Cohen and Boyer showed that DNA can be cut with restriction enzymes and reproduced by inserting the recombinant DNA into *Escherichia coli*.

1975 A moratorium on recombinant DNA experiments was called for at an international meeting at Asilomar, California, where scientists urged the government to adopt guidelines regulating recombinant DNA experimentation. The scientists insisted on the development of "safe" bacteria and plasmids that could not escape from the laboratory.

1975 Kohler and Milstein fused cells together to produce **monoclonal antibodies**.

1976 Herbert Boyer and Robert Swanson founded Genentech, Inc., a biotechnology company dedicated to developing and marketing products based on recombinant DNA technology.

1976 J. Michael Bishop and Harold Varmus, virologists at UCSF, showed that oncogenes appear on animal chromosomes, and alterations in their structure or expression can result in cancerous growth.

1976 The NIH released the first guidelines for recombinant DNA experimentation. The guidelines restricted many categories of experiments.

1977 Genentech, Inc., reported the production of the first human protein manufactured in a bacteria: somatostatin, a human growth hormone-releasing inhibitory factor. **For the first time, a synthetic, recombinant gene was used to clone a protein. Many consider this to be the advent of the Age of Biotechnology.**

1977 Sixteen bills were introduced in Congress to regulate recombinant DNA research. The bills called for the development of bacteria and plasmids that could be prevented from escaping from the laboratory environment. None of the bills passed.

1977 Bill Rutter and Howard Goodman isolated the gene for rat insulin.

1977 Walter Gilbert and Allan Maxam at Harvard University devised a method for sequencing DNA using chemicals rather than enzymes.

1978 Genentech, Inc. and The City of Hope National Medical Center announced the successful laboratory production of human insulin using recombinant DNA technology.

1978 Harvard researchers used genetic engineering techniques to produce rat insulin.

1978 Stanford University scientists successfully transplanted a mammalian gene.

1978 Studies by David Botstein and others found that when a restrictive enzyme is applied to DNA from different individuals, the resulting sets of fragments sometimes differ markedly from one person to the next. Such variations in DNA are called **restriction fragment length polymorphisms**, or RFLPs, and they are extremely useful in genetic studies.

1979 William J. Rutter's lab at UCSF cloned a coat protein of the virus that causes hepatitis B.

1979 John Baxter reported cloning the gene for human growth hormone.

1980 The U.S. Supreme Court ruled in the Chakrabarty case that genetically altered life forms can be patented. This ruling opened up enormous possibilities for commercially exploiting genetic engineering, which until that point had rested solely on the ability of companies to protect trade secrets.

1980 Kary Mullis and others at Cetus Corporation in Berkeley, California, invented a technique for multiplying DNA sequences in vitro by, the polymerase chain reaction (PCR). PCR has been called the most revolutionary new technique in molecular biology in the 1980s. Cetus patented the process, and in the summer of 1991 sold the patent to Hoffman-La Roche, Inc. for $300 million.

1981 Genentech, Inc. cloned interferon gamma.

1981 Bill Rutter and Pablo Valenzuela published a report in *Nature* on a yeast expression system to produce the hepatitis B surface antigen.

1981 Scientists at Ohio University produced the first transgenic animals by transferring genes from other animals into mice.

1981 Mary Harper and two colleagues mapped the gene for insulin. That year, mapping by *in situ* hybridization became a standard method.

1981 - 1982 Congressman Al Gore held a series of hearings on the relationship between academia and commercialization in the arena of biomedical research. He focused on the effect that the potential for huge profits from intellectual property and patent rights could have on the research environment at universities. Jonathan King, a professor at MIT speaking at the Gore hearings, reminded the biotech industry that "the most important long-term goal of biomedical research is to discover the causes of disease in order to prevent disease."

1982 Genentech, Inc. received approval from the Food and Drug Administration to market genetically engineered human insulin.

1982 Applied Biosystems, Inc. introduced the first commercial gas phase protein sequencer, dramatically reducing the amount of protein sample needed for sequencing.

1982 Lindow requested government permission to test genetically engineered bacteria to control frost damage to potatoes and strawberries.

1982 Michael Smith at the University of British Columbia, Vancouver, developed a **procedure for making precise amino acid changes anywhere in a protein**.

1982 Richard Goldstein and Richard Novick called for the prohibition of the use of RNA technologies in the development of biological weapons.

1983 Syntex Corporation received FDA approval for a monoclonal antibody-based diagnostic test for *Chlamydia trachomatis*.

1983 Stanford Research Institute International filed for a patent for an *E. coli* expression vector.

1983 Jay Levy's lab at UCSF isolated the AIDS virus (human immunodeficiency virus, HIV) at almost the same moment it was isolated at the Pasteur Institute in Paris and at the NIH.

1983 U.S. patents were granted to companies genetically engineering plants.

1983 Marvin Carruthers at the University of Colorado devised a method to construct fragments of DNA of predetermined sequence from five to about 75 base pairs long. He and Leroy Hood at the California Institute of Technology invented instruments that could make such fragments automatically.

1984 Cal Bio scientists described in *Nature* the isolation of a gene for anaritide acetate, which helps to regulate blood pressure and control salt and water excretion.

1984 Stanford University received a product patent for prokaryote DNA.

1984 Chiron Corp. announced the first cloning and sequencing of the entire human immunodeficiency virus (HIV) genome.

1984 Charles Cantor and David Schwartz developed pulsed-field gel electrophoresis.

1985 Axel Ullrich reported the sequencing of the human insulin receptor in *Nature*. Bill Rutter's UCSF team described the sequencing in *Cell* two months later.

1985 Cal Bio cloned the gene that encodes human lung surfactant protein, a major step toward reducing a premature birth complication.

1985 *Science* reported Cetus Corporation's GeneAmp **polymerase chain reaction (PCR) technology**, which could generate billions of copies of a targeted gene sequence in only hours.

1985 Genetically engineered plants resistant to insects, viruses, and bacteria were field tested for the first time.

1985 The NIH approved guidelines for performing experiments in gene therapy on humans.

1985 Genetic Sciences (AGS) surreptitiously performed the first deliberate release experiment, injecting genetically engineered microbes into trees growing on the company's roof, while waiting for approval from the EPA to conduct a different deliberate release experiment involving strawberry plants.

1986 UC Berkeley chemist Peter Schultz described how to combine antibodies and enzymes (creating "abzymes") to create pharmaceuticals.

1986 A regiment of scientists and technicians at Caltech and Applied Biosystems, Inc., invented the automated DNA fluorescence sequencer.

1986 The FDA granted a license for the first recombinant vaccine (for hepatitis) to Chiron Corp.

1986 The EPA approved the release of the first genetically engineered crop, gene-altered tobacco plants.

1987 Genentech received FDA approval to market rt-PA (genetically engineered tissue plasminogen activator) to treat heart attacks.

1987 Calgene, Inc. received a patent for the tomato polygalacturonase DNA sequence, used to produce an antisense RNA sequence that can extend the shelf-life of fruit.

1987 Advanced Genetic Sciences, Inc. conducted a field trial of a recombinant organism, a frost inhibitor, on a Contra Costa County strawberry patch.

1987 Maynard Olson and colleagues at Washington University invented "yeast artificial chromosomes," or YACs, expression vectors for large proteins.

1988 Philip Leder and Timothy Stewart, molecular geneticists at Harvard, introduced the "Harvard Mouse"—a line of genetically engineered laboratory mice. They were the first to win a patent for a mammal in the U.S.

1988 SyStemix Inc. received a patent for the SCIDHU Mouse, an immune-deficient mouse with a reconstituted human immune system. The mouse was engineered for AIDS research.

1988 Genencor International, Inc. received a patent for a process to make bleach-resistant protease enzymes to use in detergents.

1989 UC Davis scientists developed a recombinant vaccine against the deadly rinderpest virus, which had wiped out millions of cattle in developing countries.

1990 UCSF and Stanford University were issued their 100th recombinant DNA patent license. By the end of fiscal 1991, both campuses had earned $40 million from the patent.

1990 The first successful field trial of genetically engineered cotton plants was conducted by Calgene Inc. The plants had been engineered to withstand use of the herbicide Bromoxynil.

1990 The FDA licensed Chiron's hepatitis C antibody test to help ensure the purity of blood bank products.

1990 Michael Fromm, molecular biologist at the Plant Gene Expression Center, reported the stable transformation of corn using a high-speed gene gun.

1990 Mary Claire King, epidemiologist at UC-Berkeley, reported the discovery of the gene linked to breast cancer in families with a high degree of incidence before age 45.

1990 GenPharm International, Inc. created the first transgenic dairy cow. The cow was used to produce human milk proteins for infant formula.

1990 A four-year-old girl suffering from ADA deficiency, an inherited disorder that destroys the immune system, became the **first human recipient of gene therapy**. The therapy appeared to work, but set off a fury of discussion of ethics both in academia and in the media.

1990 The **Human Genome Project**, the international effort to map all of the genes in the human body, was launched. Estimated cost: $13 billion.

1991 The celebrated reference work "Mendelian Inheritance in Man," was made available through an on-line computer network. The catalogue lists some 5,600 genes known or thought on good evidence to be inherited in Mendelian patterns.

ANNEX B:
Selected Biotechnology Terms

Antigen – a chemical, protein, or microorganism that is recognized by, and attaches to an antibody (usually uniquely to a specific antibody)

Antibiotic – any of a variety of substances, usually obtained from microorganisms, that inhibit the growth of or destroy certain other microorganisms. Effective in the treatment and prevention of bacterial and rickettsial diseases. Ineffective against viral diseases. Examples include tetracycline, ciproflaxin, and erythromycin, among many others.

Antibody – an immunoglobulin that specifically recognizes and binds to an antigenic determinant on an antigen. Antibodies destroy or weaken bacteria and neutralize organic poisons, thus forming the basis of immunity.

Bioregulators – chemicals or enzymes that control physiological functions, such as pain, sleep, or mood.

Cloning – the process of preparing a largely identical group of organisms, cells, viruses, or nucleic acid molecules (including genes or gene fragments) descending from a single common ancestor

***Escherichia coli* (*E. coli*)** – a common type of bacteria found in the human intestine and aids in digestion. Many strains of the *E. coli* bacteria in gene splicing.

Gene Amplification – the creation of extra, functional copies of genes in a cell or organism.

Gene Expression – the combination of decoding the genetic information and synthesis of the gene product. Gene expression proceeds by two major steps—transcription and translation. *Transcription* is the synthesis of different types of RNA molecules (particularly messenger RNA, mRNA) according to the specific information of the gene transcribed. *Translation* is the synthesis of polypeptides using mRNA as a template which is encoded by polypeptide encoding genes.

Gene Mapping – locating the positions of the genes on the chromosomes of a particular organism

Gene Splicing – see polymerase chain reaction

Genome – the section of DNA that carries the complete set of genetic information for a virus, cell, or organism.

Monoclonal Antibodies – one of a group of identical antibodies able to react with on and the same antigen. Produced by a clone of engineered antibody-producing ("hybridoma") cells obtained by fusion of immortal tumor cells with stimulated lymphocytes.

Mutagen – an agent that increases the rate of mutation by causing changes in the nucleotide sequences of DNA (for example, carcinogens)

Physiologically Active Compounds (PACs) – (see also bioregulators.) Endogenous mammalian compounds such as hormones, neurotransmitters, and neuropeptides. Examples include adenosine triphosphate (ATP), corticotropin releasing factor, dynorphin, enkephalin, glutamate, morphine modulatory peptide, N-acetyl-aspartyl-glutamate, nitric oxide, norepinephrine, serotonin, substance P, tumor necrosis factor, vasoactive intestinal peptide.

Polymerase Chain Reaction (PCR) – a method for the selective amplification of a DNA bas sequence using heat-stable polymerase and two 20-base primers. Because the newly synthesized DNA strands can serve as templates for the same primer sequences successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. PCR can also be used to detect the existence of the defined sequence in the DNA sample.

Recombinant DNA – a DNA molecule made up of sequences that are not normally joined together, created by the process of cleaving an rejoining different DNA strands.

Transcription (T_C) – see Gene Expression

Translation (T_L) – see Gene Expression

Vaccine – a preparation of dead or attenuated pathogens, or of derived antigenic determinants, that is used to induce formation of antibodies or immunity against the pathogen. May be effective against specific viruses, bacteria, rickettsia, *etc*.

Vectors – Also, expression vectors. A vehicle for moving DNA from one cell to another, such as a plasmid into which foreign DNA can easily be inserted and which will be efficiently taken up by the host cell. Can act as a carrier molecule for the construction of recombinant DNA.