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SECTION III

BIOLOGICAL WEAPONS TECHNOLOGY

SECTION 3—BIOLOGICAL WEAPONS TECHNOLOGY

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BACKGROUND

Biological agents are naturally occurring microorganisms (bacteria, viruses, fungi) or toxins that can cause disease and death in a target population. They can also attack the food supply and/or materiel of a nation. Biological weapons (BW) which project, disperse, or disseminate biological agents have two characteristics that enhance their effectiveness as weapons: (1) biological agents, other than toxins, reproduce and, therefore, a small amount of infectious agent can cause disease; (2) biological agents, other than toxins, usually require an incubation period of hours to days to manifest signs of exposure so the affected soldier is not certain whether a biological agent attack has occurred until illness sets in. The uncertainty can compromise unit cohesion and weaken U.S. force superiority.

The United States has forsworn the use of biological weapons and has developed a strategy of offensive strike power by other means, coupled with biological defense capability, as a suitable deterrent to potential adversaries. A nation, subnational group, or organization, or even an individual, determined to construct a biological weapon and release the agent can, with minimal financial resources and infrastructure, produce an effective weapon. Small amounts of biological material are sufficient because of the reproductive nature of microorganisms. The availability of small amounts of biological organisms, including those listed by the Australia Group (AG), in culture collections provides a major resource for such determined entities. All of these stocks are also available from natural sources, such as soil samples and infected rodents. In addition to naturally occurring organisms, genetically modified organisms may be used as biological agents. Some organisms exist primarily in repositories and may be used as biological agents (Variola Virus). It is estimated that between 10 and 10,000 virulent organisms of the AG agents are sufficient to cause illness in one individual. The number of organisms required is a function of the specific agent and the means of delivery. The delivery of a limited amount of a biological agent might be militarily significant if the agent is released in a contained environment (e.g., a closed building, submarine, or surface vessel).

Highlights

- Biological weapons are unique because they are made up of pathogenic organisms that can reproduce and cause infection (and death) in a large number of hosts.
- It takes hours to days for symptoms of exposure to appear.
- Biological weapons are relatively inexpensive to produce.
- All of the equipment used to produce biological agents is dual use, with applications in the pharmaceutical, food, cosmetic, and pesticide industries.
- Dissemination and dispersion are key to the effective employment of biological weapons.
- Many toxic organisms are subject to destruction by external forces (e.g., sunlight, explosives).

There are aspects that make biological weapons agents unique and different from all other weapon systems. Whereas a subnational group would be required to have a significant infrastructure to develop nuclear devices, it would be less complicated to make biological agents. Moreover, the biological agent could be a strategic and disorganizing threat because of its ability to reproduce and the delayed manifestation of symptoms. Those delivering BW could be protected by active or passive immunization or by well-designed protective masks to protect the respiratory system from aerosols, the primary delivery mechanism.

An additional concern is the relative low cost required for the production and the ease of deployment of biological agents by subnational groups and organizations for biomedical, pharmaceutical, and food production. All of the equipment used to produce biological agents is dual use.

Because biological agents reproduce, only small amounts of a starter organism are needed. The use of appropriate growth media or nutrients in a cell culture system of 100 liters, or of four passes through a 25-liter system, can generate sufficient agent to infect numerous targets in a contained area (e.g., subway, contained office building). Other weapons of mass destruction (WMD) require the purchase of large amounts of precursor or of fissile material to achieve threat capability. The self-generation of the biological agent is a unique element of this WMD.

Biologically derived toxins also present a threat. The recent apprehension in the United States of an individual citizen who produced large quantities of the toxin ricin is an example of the danger related to the production of toxin WMDs by small groups. As with other chemical agents, the toxins do not reproduce and, therefore, represent a threat that differs quantitatively from biological agents.

1. History of Biological Weapons

Crude forms of biological warfare have been employed since 300 B.C., when the decaying corpses of animals and humans were placed near water and food supplies of adversaries. Over the years, different diseases, including plague and smallpox, were used as the agent. Catapults were one vehicle for introduction of the infected tissue. Other vehicles, including blankets, have been employed to transmit smallpox to a target population.

World War I saw the development of biological warfare strategies. Cholera and plague were thought to be used in Italy and Russia while anthrax was presumably used to infect animals in Romania. A consequence of such events was the 1925 Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous, or Other Gases, and of Bacteriological Methods of Warfare—known as the Geneva Protocol. This protocol banned the use of biological agents in warfare but not research, development, production, or stockpiling of such agents.

With the advent of World War II, rapid developments occurred in biological warfare capability in the United States and other nations. In February 1942, the U.S. National Academy of Sciences established a Biological Warfare Committee, chaired by Edwin B. Fred of the University of Wisconsin. The administration of the biological warfare effort was placed under civilian supervision: Dr. George Merck directed the advisory group, and Ira Baldwin of the University of Wisconsin became the scientific director. In 1943, Fort Detrick, Maryland, became the site of these activities, as Camp Detrick. In Canada, Sir Fredrick Banting, Dr. J.R. Collys, and Dr. Charles Best led the biological warfare capability effort.

The technologies examined at Fort Detrick included pathogen identification, modes of transmission, infection, detection, public health measures, containment, rapid drying of organisms, and packing for delivery. In 1969, President Nixon stated that the U.S. unilaterally renounced biological warfare. Biological weapon stockpiles and their associated munitions were destroyed following the preparation of an environmental impact statement and review by both federal and state authorities and the public. Low targeting capability, the potential for catastrophic outcome on civilian populations, and public antipathy to biological weaponry were factors in the renunciation of biological warfare. In 1972, there was international agreement to the Convention of the Prohibition of the Development, Production, and Stockpiling of Bacteriological and Toxin Weapons and their Destruction [Biological Weapons Convention (BWC)]. Concern over USSR compliance with the Convention arose with the sudden outbreak of anthrax cases in Sverdlovsk (now Ekaterinenberg) in 1979.

The early 1980's saw renewed discussion of the utility of biological weapons as strategic weapons. For example, information became publicly available concerning studies of biological agents in Japan and the studies on the effects of infectious agents on human subjects in Harbin, Manchuria, during World War II. The number of infectious agents used on human populations was about 25 (e.g., plague, typhus, smallpox, tularemia, gas gangrene, tetanus, cholera, anthrax, tick encephalitis). In 1941, the Japanese deployed plague-infected fleas in Hunan Province, resulting in the death of several hundred persons. The difficulty encountered by the Japanese was the development of an effective delivery system.

In recent years, newly emerging infectious diseases have complicated the picture. They include AIDS, prion disorders, and several hemorrhagic fevers such as Ebola. These diseases and the possible reduction in immunocompetence have fostered an increased role of the United States and international agencies in monitoring disease outbreaks. Several federal agencies in the United States are responsible for the health and protection of the population, including military personnel, from infectious diseases. The civilian agencies include the National Institutes entities that address health care issues of primary importance to the defense community: Walter Reed Army Institute of Research, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), and the Naval Medical Research Units.

2. Recent Developments Affecting Biological Warfare Capability

The introduction of modern biotechnology during the past 25 years has markedly changed the qualitative and quantitative impact that biological warfare, or the threat of such warfare, can have on military forces and urban communities. This new technology provides the potential capability of (1) developing biological agents that have increased virulence and stability after deployment; (2) targeting the delivery of organisms to populations; (3) protecting personnel against biological agents; (4) producing, by genetic modification, pathogenic organisms from non-pathogenic strains to complicate detection of a biological agent; (5) modifying the immune response system of the target population to increase or decrease susceptibility to pathogens; and (6) producing sensors based on the detection of unique signature molecules on the surface of biological agents or on the interaction of the genetic materials in such organisms with gene probes. The specific technologies used in realizing these capabilities include (1) cell culture or fermentation; (2) organism selection; (3) encapsulation and coating with straight or crosslinked biopolymers; (4) genetic engineering; (5) active or passive immunization or treatment with biological response modifiers; (6) monoclonal antibody production; (7) genome data bases, polymerase chain reaction equipment, DNA sequencers, and the rapid production of gene probes; and (8) the capability of linking gene probes and monoclonal antibodies on addressable sites in a reproducible manner.

New technologies related to biological warfare are emerging rapidly. The technology of monoclonal antibody production has existed only since 1975, while the technology of genetic engineering has existed since the 1980's. Technology for

sequencing the genomes of organisms has changed so dramatically that the rate of sequencing has increased by several orders of magnitude since 1994. All of these reflect the enormous change in information databases and in technology including biotechnology, computer equipment, processes, and networking of research teams. Information that will emerge from the human genome effort is likely to increase our understanding of the susceptibilities of different populations to disease and stresses of various sources. Such information may increase the proliferation of BW agents, particularly in areas with active ethnic rivalries, and lead to a new variant of ethnic cleansing.

The rapid rate of development reflects to some degree the national and international investment in this technology. The level of federal spending in the United States in the entire biotechnology area during 1994 approximated 4 billion dollars. The private sector invested approximately 7 billion dollars during the same year. This investment and the rate of information accrual indicates that biological technology that can be used for peaceful and military purposes is increasing in capability at a rate exceeding most other technologies. The pharmaceutical industry is relying on biotechnology for new therapeutic products to improve prophylaxis and therapy for many different diseases and is concerned that these new technologies not fall into the hands of potential adversaries.

Figure 3.0-1 portrays graphically the explosive growth of applicable biotechnologies. The illustration was prepared from a broad field of knowledge and applications, which, in aggregate, are doubling every 18 months. Examples of sustained geometric growth include monoclonal antibodies, combinatorial chemistry, and gene probes, which are explained below.

- Monoclonal Antibodies - In the early 1970's, Kohler and Milstein developed a procedure to produce antibodies for a single antigenic epitope. An epitope is the region of a molecule that initiates the production of a single antibody species. The dimensions of an epitope approximate a surface area 50×50 Angstroms. These antibodies are called monoclonal antibodies. With quality control, these antibodies can be produced in gram quantities in a highly reproducible manner, and therefore, they are suited for industrial uses. The industries currently using monoclonal antibodies include medical diagnostics, food, environmental protection, and cosmetics.

- Combinatorial Chemistry - This is a technique for rapidly synthesizing large numbers of peptides, polynucleotides, or other low molecular weight materials. These materials are synthesized on a solid-state matrix and in an addressable form so that materials of known sequence can be accessed readily. The materials can function as receptors, pharmaceuticals, or sensor elements. The technique, developed by Merrifield in the 1970's, has been essential for the growth of combinatorial chemistry.

- Gene Probes - These are polynucleotides that are 20–30 units long, under stringent conditions, complementary nucleic acid fragments characteristic of biological agents. These units provide the basis of rapid detection and identification.

OVERVIEW

This section of the MCTL is concerned with technologies related to the development, integration and deployment of biological weapons. The infectious organisms discussed are those identified by the AG (see Figure 3.0-2). The AG list does not include every known organism that could be used in a biological weapon. Toxins will be considered in the biological weapons section consistent with the AG and the BWC of 1972. Several aspects of biological warfare will be covered: (1) the identity of the biological organism or toxins; (2) equipment and materials necessary for the production, containment, purification, quality control, and stabilization of these agents; (3) the technologies for the dissemination and dispersion of biological agents; (4) equipment for detection, warning, and identification of biological agents; and (5) individual and collective biological defense systems.

RATIONALE

Biological weapons are unique because the effects from pathogenic organisms, except toxins, are not seen for hours to days after dissemination. If adequate detection devices are not available, the first indication of a biological weapon attack could be symptoms in target personnel. At this point, treatment prophylaxis and therapy is often ineffective. In addition, incapacitated troops require tremendous logistical support (four or five medical corpsmen and associated personnel for each ill person); thus, incapacitants may be preferable to lethal agents. Also, besides deaths caused by infectious agents, the psychophysical damage suffered by troops who believe they have been exposed to a biological attack could markedly impair combat functions. The perception is almost as significant as the reality. The affected soldier is not certain whether a biological attack has occurred and could be psychologically, if not physically, impaired.

The biological technology industry is information intensive rather than capital intensive. Data on technologies involved in biological production are widely available in the published literature. These technologies are dual use, with applications in the pharmaceutical, food, cosmetic, and pesticide industries. New technologies, such as genetic engineering, are more likely to affect fabrication, weaponization, or difficulty of detection than to produce a "supergerm" of significantly increased pathogenicity.

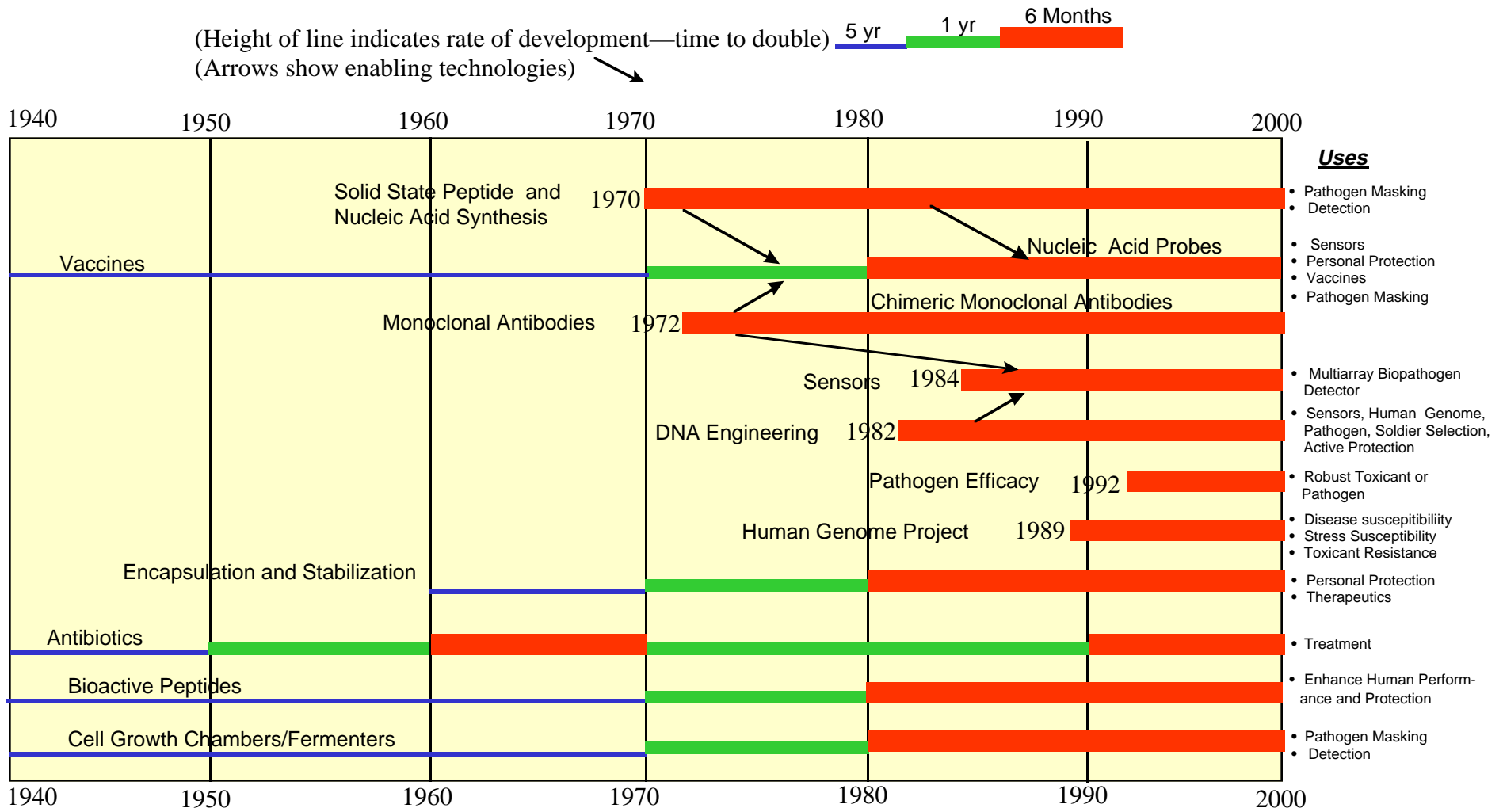


Figure 3.0-1. Progress in Applicable Biotechnologies

Viruses	
V1.	Chikungunya virus
V2.	Congo-Crimean haemorrhagic fever virus
V3.	Dengue fever virus
V4.	Eastern equine encephalitis virus
V5.	Ebola virus
V6.	Hantaan virus
V7.	Junin virus
V8.	Lassa fever virus
V9.	Lymphocytic choriomeningitis virus
V10.	Machupo virus
V11.	Marburg virus
V12.	Monkey pox virus
V13.	Rift Valley fever virus
V14.	Tick-borne encephalitis virus (Russian spring-summer encephalitis virus)
V15.	Variola virus
V16.	Venezuelan equine encephalitis virus
V17.	Western equine encephalitis virus
V18.	White pox
V19.	Yellow fever virus
V20.	Japanese encephalitis virus

Rickettsiae	
R1.	Coxiella burnetti
R2.	Bartonella quintana (Rochlimea quintana, Rickettsia quintana)
R3.	Rickettsia prowasecki
R4.	Rickettsia rickettsii

Bacteria	
B1.	Bacillus anthracis
B2.	Brucella abortus
B3.	Brucella melitensis
B4.	Brucella suis
B5.	Chlamydia psittaci
B6.	Clostridium botulinum
B7.	Francisella tularensis
B8.	Burkholderia mallei (pseudomonas mallei)
B9.	Burkholderia pseudomallei (pseudomonas pseudomallei)
B10.	Salmonella typhi
B11.	Shigella dysenteriae
B11.	Vibrio cholerae
B13.	Yersinia pestis

Genetically Modified Microorganisms	
G1.	Genetically modified microorganisms or genetic elements that contain nucleic acid sequences associated with pathogenicity and are derived from organisms in the core list.
G2.	Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins in the core list or their subunits.

Toxins	
T1.	Botulinum toxins
T2.	Clostridium perfringens toxins
T3.	Conotoxin
T4.	Ricin
T5.	Saxitoxin
T6.	Shiga toxin
T7.	Staphylococcus aureus toxins
T8.	Tetrodotoxin
T9.	Verotoxin
T10.	Microcystin (Cyanginosin)
T11.	Aflatoxins

Viruses (Warning List)	
WV1.	Kyasanur Forest virus
WV2.	Louping ill virus
WV3.	Murray Valley encephalitis virus
WV4.	Omsk haemorrhagic fever virus
WV5.	Oropouche virus
WV6.	Powassan virus
WV7.	Rocio virus
WV8.	St Louis encephalitis virus

Bacteria (Warning List)	
WB1.	Clostridium perfringens
WB2.	Clostridium tetani
WB3.	Enterohaemorrhagic Escherichia coli, serotype O157 and other verotoxin- producing serotypes
WB4.	Legionella pneumophila
WB5.	Yersinia pseudotuberculosis

(cont'd)

Figure 3.0-2. Australia Group Biological Agents

<p align="center">Genetically Modified Microorganisms</p> <p>WG1. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences associated with pathogenicity and are derived from organisms in the warning list.</p> <p>WG2. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins in the warning list or their subunits.</p>	<p align="center">Animal Pathogens (cont'd)</p> <p><u>Viruses (cont'd):</u> AV11. Porcine enterovirus type 9 (synonym: Swine vesicular disease virus) AV12. Rinderpest virus AV13. Sheep pox virus AV14. Teschen disease virus AV15. Vesicular stomatitis virus</p> <p><u>Bacteria:</u> AB3. Mycoplasma mycoides</p> <p><u>Genetically Modified Microorganisms:</u> AG1. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences associated with pathogenicity and are derived from animal pathogens on the list.</p>	<p align="center">Plant Pathogens (cont'd)</p> <p><u>Fungi (cont'd):</u> PF5. Puccinia striiformis (syn. Pucciniaglomerum) PF6. Pyricularia grisea/Pyricularia oryzae</p> <p><u>Genetically Modified Microorganisms:</u> PG1. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences associated with pathogenicity derived from the plant pathogens on the list.</p>
<p align="center">Toxins (Warning List)</p> <p>WT1. Abrin WT2. Cholera toxin WT3. Tetanus toxin WT4. Trichothecene mycotoxins WT5. Modecin WT6. Volkensin WT7. Viscum Album Lectin 1 (Viscumin)</p>	<p align="center">Plant Pathogens</p> <p><u>Bacteria:</u> PB1. Xanthomonas albilineans PB2. Xanthomonas campestris pv. citri</p> <p><u>Fungi:</u> PF1. Colletotrichum coffeanum var. virulans (Colletotrichum kanawae) PF2. Cochliobolus miyabeanus (Helminthosporium oryzae) PF3. Microcyclus ulei (syn. Dothidella ulei) PF4. Puccinia graminis (syn. Puccinnia graminis f. sp. tritici)</p>	<p align="center">Awareness Raising Guidelines</p> <p><u>Bacteria:</u> PWB1. Xanthomonas campestris pv. oryzae PWB2. Xylella fastidiosa</p> <p><u>Fungi:</u> PWF1. Deuterophoma tracheiphila (syn. Phoma tracheiphila) PWF2. Monilia rorei (syn. Moniliophthora rorei)</p> <p><u>Viruses:</u> PWV1. Banana bunchy top virus</p> <p><u>Genetically Modified Microorganisms:</u> PWG1. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences associated with pathogenicity derived from the plant pathogens identified on the awareness raising list.</p>
<p align="center">Animal Pathogens</p> <p><u>Viruses:</u> AV1. African swine fever virus AV2. Avian influenza virus AV3. Bluetongue virus AV4. Foot and mouth disease virus AV5. Goat pox virus AV6. Herpes virus (Aujeszky's disease) AV7. Hog cholera virus (synonym: Swine fever virus) AV8. Lyssa virus AV9. Newcastle disease virus AV10. Peste des petits ruminants virus</p>		

Figure 3.0-2. Australia Group Biological Agents (cont'd)

While laboratory-scale capability for production of biological agents is sufficient for achieving most terrorist purposes, large-scale production for military purposes can be achieved easily in dual-use facilities. All of the equipment needed for large-scale production of offensive biological agents is dual use and available on the international market. Although a typical vaccine plant costs in excess of \$50 million, a less elaborate fermentation plant that could produce biological agents could be built for less than \$10 million.

If disseminated properly, only a small amount of biological agent is needed to infect numerous people. Proper dissemination, however, is a non-trivial problem because the agent must be dispersed in 1 to 10 micron particles and be inhaled by the target population. Symptoms normally take hours to days to appear. Detection is key to implementation of protective measures. Since biological organisms are living, they have the potential to reproduce. They can continue to affect people for extended periods of time. However, they are subject to being negated by sunlight and the environment, but most can be effectively stabilized against adverse environmental effects. Stress from explosive dissemination and/or missile firing can reduce efficiency to about the 5-percent level, which is why aerosol dissemination by pressurized gases was adopted by munition designers in the old U.S. program. Dissemination efficiencies of up to 70 percent were achieved, with 30 to 50 percent being produced routinely. Vaccines can be produced to defend against biological agent use; however, to produce the vaccine, the organism being employed by an adversary must be known.

Although some of the proliferation concerns for biological weapons are similar to those for other WMD, some concerns are unique. The unique features include containment of the agent during production, stabilization and dispersion of the agents, detection, identification, and warning. All these aspects are important because biological agents are relatively easy to hide. The diffusion of information, technologies, and raw materials associated with biological and pharmaceutical processing are almost always dual use and, therefore, raise non-proliferation issues.

Because of the low financial costs of acquiring equipment for biological agent production, the implications for the proliferation of production and dispersion are clear: developing nations can attack targets effectively with biological agents. Defensive technologies are of interest because changes in vaccine production or other self-protection measures could presage an offensive attack. Stabilization and dispersion are proliferation concerns because these technologies increase the efficacy of biological agents. Detection, identification, and warning technologies can be used to support efforts to mask the presence of biological agents even though these technologies do not pose a direct threat.

FOREIGN TECHNOLOGY ASSESSMENT (See Figure 3.0-3)

Most industrialized nations manufacture equipment and materials that can be used for the production, containment, purification, quality control, and stabilization of biological agents and for their dissemination and dispersion. Most developed nations manufacture the equipment for identifying these agents, but the means for detection and warning are less readily available. All these technologies are dual use, with applications in the pharmaceutical, food, cosmetic, and pesticide industries. The AG group of biological agents are readily available in the natural environment and from culture collections in the industrialized and in some developing nations. The recent outbreaks of Ebola in Africa and Hanta (Hantaan) virus infections in Asia and North and South America are evidence of occurrence in the natural environment. In addition, these organisms can be obtained from national collections [e.g., American Type Culture Collection (ATCC) and European collection]. The ATCC and European collections do not necessarily share information.

Many collections of organisms recognized as potential biological agents and included in the AG list exist throughout the world and are made available with minimal monitoring of use or transport. This is particularly the case in the open societies of the United States, Europe, and Japan, as was documented in 1995 by a case occurring in Ohio. The nutrients, growth media, and small-size fermenters are readily available.

Country	Sec 3.1 Biological Material Production	Sec 3.2 Stabilization, Dispersion and Weapons Testing	Sec 3.3 Detection, Warning, and Identification	Sec 3.4 Biological Defense Systems
Australia ¹	◆◆	◆◆	◆◆	◆◆◆
Austria ¹	◆	◆◆	◆◆◆	◆◆◆
Belgium ¹	◆◆	◆◆	◆◆	◆◆
Brazil	◆◆	◆◆	◆	◆
Bulgaria	◆	◆◆	◆◆	◆◆◆
Canada ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
China	◆◆◆	◆◆◆	◆◆◆	◆◆◆
Cuba	◆◆	◆◆	◆◆	◆
Czech Republic ¹	◆◆	◆◆◆	◆◆◆	◆◆◆◆
Denmark ¹	◆◆	◆◆	◆	◆◆
Egypt	◆	◆◆	◆	◆◆
Finland ¹	◆◆◆	◆◆◆	◆◆◆	◆◆◆
France ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
Germany ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
Greece ¹	◆	◆	◆	◆◆
Hungary ¹	◆◆◆	◆◆	◆◆◆	◆◆◆◆
India	◆◆◆	◆◆◆	◆◆	◆◆
Iran	◆◆	◆◆	◆	◆◆
Iraq	◆◆◆	◆◆	◆	◆◆
Israel	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
Italy ¹	◆◆◆	◆◆	◆◆	◆◆
Japan ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
Korea (North)	◆◆	◆◆	◆	◆◆
Korea (South) ¹	◆◆◆	◆◆	◆◆	◆◆◆
Libya	◆◆	◆	◆	◆
Netherlands ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
Norway ¹	◆◆◆	◆◆◆	◆◆	◆◆
Pakistan	◆◆	◆◆	◆◆	◆◆◆
Poland ¹	◆◆	◆◆	◆◆	◆◆◆
Romania ¹	◆◆	◆	◆◆	◆◆◆
Russia	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
Slovak Republic ¹	◆◆	◆◆	◆◆◆	◆◆◆
South Africa	◆◆	◆◆	◆◆◆	◆◆◆
Spain ¹	◆◆	◆	◆	◆
Sweden ¹	◆◆◆◆	◆◆◆	◆◆◆◆	◆◆◆◆
Switzerland ¹	◆◆◆◆	◆◆◆	◆◆◆	◆◆◆
Syria	◆◆	◆◆	◆	◆
Turkey	◆◆	◆◆		
Ukraine	◆◆◆◆	◆◆◆	◆◆◆	◆◆◆
United Kingdom ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆
United States ¹	◆◆◆◆	◆◆◆◆	◆◆◆◆	◆◆◆◆

¹ Indicates that the nation is a member of the Australia Group (AG).

Legend: Sufficient Technologies Capabilities: ◆◆◆◆ exceeds sufficient level ◆◆◆ sufficient level ◆◆ some ◆ limited

Because two or more countries have the same number of diamonds does not mean that their capabilities are the same. An absence of diamonds in countries of concern may indicate an absence of information, not of capability. The absence of a country from this list may indicate an absence of information, not capability.

Figure 3.0-3. Biological Weapons Foreign Technology Assessment Summary

SECTION 3.1—BIOLOGICAL MATERIAL PRODUCTION

OVERVIEW

The previous section addressed the various organisms that might be selected for production (The AG Biological Agents). This section addresses the production of the organisms, including procedures such as culture, fermentation, viral reproduction, etc.; the stabilization of the organisms; and specific equipment used in the manufacturing process.

The stages involved in the production of biological agents include selection of the organisms, large-scale production of organisms from small starter cultures, and stabilization of the organisms. The list of biological organisms and toxin products that are of concern as biological agents is derived from the AG consensus.

The design of a production facility provides important information regarding whether the facility is intended to produce pharmaceutical grade products or biological weapon grade materials. Relevant design elements include containment, purification equipment, sterilization equipment, and ventilation and filtration systems.

The design of a biochemical processing plant is an important signal of covert biological agent production. Containment of the biological material during processing is of special interest. There is a clear distinction between processing materials for biological or toxin agent weaponization and processing protective agents to be used for countermeasures or personnel performance enhancement. For the production of biological agents for offensive military activities, the processing containment requirement is to protect the environment from the agent because of its infectious nature. For the production of biomaterials, such as vaccines, biological response modifiers, antibiotics, and anti viral agents, for defensive military activities, the containment requirement is to protect the processed biomaterial from contaminating materials in the environment. Effectiveness of countermeasures is enhanced by achieving high levels of purity and cleanliness in the product before it is administered to friendly personnel. By contrast, an unpurified biological agent that will be used in BW is generally more stable than the purified agent that is needed to produce vaccines and biological response modifiers (BRMs). Consequently, a proliferant does not require a high level of purity if production is for BW use only.

Generation of biological agents requires fermenters or single cell production capabilities with operational conditions identified in the MCTL, including smooth, highly polished stainless steel surfaces, self-containment capability, and negative pressure conditions. The primary difference between the production requirements for biological weapons and non-military commercial purposes lies in containment and contamination. During biological agent production, efforts are generally made to avoid contaminating the environment with the organism. Less concern arises about the

Highlights

- Biological weapon production is similar to commercial production of biological materials.
- With the exception of toxins, biological organisms can multiply.
- Containment of the organisms is critical.
- Design of the plant can indicate covert biological agent production.

contamination of the product. Conversely, the pharmaceutical, brewing, and biotechnology industries are most concerned about protecting the purity and quality of the product. This concern is reflected in the nature of the sealing joints, positive or negative pressure chambers, and containment of venting systems.

Utilities involving clean steam, sterile air, and inert gas supply are most critical for containment in the processing of biologically based materials for human use, which must meet good manufacturing practices (GMP). Clean steam, generated from a purified water supply, must be supplied to all processing equipment having direct contact with the product to ensure sterility and prevent the influx of environmental contaminants. Steam sterilization is accomplished before product processing by direct supply to the equipment. Steam is supplied to the equipment seals (e.g., sample ports, agitator shafts, raw material addition ports) during processing as a primary barrier. Equally important is the removal of collapsed steam or condensate formed on the equipment. This prevents the formation of pockets of standing water, which promote bacterial growth, and maintains the high temperature necessary for sterilization. The collected contaminated condensate can be channeled to an area for final sterilization or inactivation before it is released into the environment. Efficient steam supply and condensate removal requires pressure regulators, pressure relief devices, venting, and the capability for free draining of all lines.

Supplying sterile, inert gases to processing equipment is a method of containment. This can protect oxygen-sensitive biomaterials and prevent aerosol generation of toxic products. Inert gases, such as nitrogen, helium, and argon, are usually supplied directly to processing equipment through sterile, in-line filters, maintaining a pressurized system or providing an inert blanket over the product in processing vessels.

To attain a higher level of containment, many bioprocessing industries have employed greater degrees of automation. Potential contamination of purified product, human exposure to toxic products or constituents, and the risk of human error are

minimized. Processing facilities make use of state-of-the-art computerized distributed control systems (ABB, Modicon, Allen Bradley Corp.), which allow automatic control, control from remote locations, and automatic data logging and trending.

Another component in bioprocessing is the design of ventilation within the primary and secondary barriers of a process area. Ventilation at primary barriers (i.e., barriers separating product from equipment operators and the rest of the processing area) is accomplished with dedicated, in-line air/gas membrane filters. Ventilation across secondary barriers requires more complicated air handling system design to allow for the maintenance of clean areas (rated by the number of particles per volume of air) and maintenance of positive or negative pressure between the processing area and the outside environment or between different processing areas in the same facility. Equipment used in these designs includes high efficiency fans and high efficiency particulate air (HEPA) filters.

The procedure used for the actual replication of an organism is a function of the organism itself. Tables 3.1-1 and 3.1-2 include several techniques, including cell culture, fermentation, viral replication, recombinant DNA, and powdering and milling. Cell culture is necessary for the reproduction of pathogenic viruses and Rickettsiae since they will not reproduce outside a living cell (e.g., chick embryo or tissue cultures). Single cell growth chambers, including fermentation, are used for the production of bacteria and bacterial toxins, although some bacteria (e.g., plague bacteria) can also be cultivated in living animals. Recombinant DNA techniques are a preferred method to produce rare animal toxins. Because of the complexity of this technique, the capability is not as widespread as the others. Powdering and milling is the technique generally used to produce BW and toxin weapons (TW) agent particles having diameters less than or equal to 10 µm, the size most effective for respiratory delivery.

RATIONALE

Figure 3.0-2 lists the naturally occurring pathogens and toxins potentially used as BW agents. Whereas the majority of these agents have no current dual-use applications, a small number do have biomedical roles other than those in vaccine production. The highly toxic botulinum toxin A, produced by *Clostridium botulinum*, shows medicinal promise in blocking involuntary muscle spasms or weakening a muscle for therapeutic purposes. Five medical uses of toxins that might be used in BW have been approved by the Food and Drug Administration. Immune protection against these agents is important because they occur naturally in some regions of the world. Toxins and pathogens that affect animals, such as anthrax, brucella, plague, and tularemia, are widespread. Vaccines are widely produced and administered. The issue of the need for the same toxic agent for either BW/TW production or countermeasure vaccine production emphasizes the dual-use nature of the technologies. Indeed, initial processing of agents and processing of their associated vaccines only differ by a few steps (e.g., the degree of purification and the type of containment used).

The qualitative and quantitative impact of biological warfare, or the threat of such warfare, on military forces and urban communities has changed markedly in the past 20 years. The production techniques described in this section have resulted in more virulent strains of organisms and the genetic modification of non-pathogenic organisms to pathogenic strains with virulent characteristics. The implications of genetic engineering for chemical and biological warfare are far-reaching. Genetic engineering provides the potential for improved virulence by the incorporation of genes (i.e., specific strands of DNA) permitting increased production of a pathogen or toxin. Thus, as much as 100 times more pathogen or toxin could be produced per cell than that which could be produced by naturally occurring strains. Cells that normally do not produce toxins may be altered to produce toxins for biological weapon development. Conversely, known pathogens or toxins may be genetically inactivated for vaccine countermeasure development. Cells can also be modified to produce antibodies directly for passive immunization against specific infectious agents. As with the human immune system, many current biowarfare detection kits depend on antibodies reacting with the antigenic surface coatings of pathogenic bacteria or viruses. Thus, modified non-pathogens can be used to mask the agent from the immune-based detector and, potentially, from the human immune system itself to increase the agent's effectiveness.

General robustness or survivability of a pathogen under the environmental stresses of temperature, ultraviolet (UV) radiation, and desiccation (drying) can also be genetically improved to promote stability during dissemination; nutrient additives are used to enhance survival of selected biological agents in aerosols. Controlled persistence of a pathogen to permit survivability under specified environmental conditions may eventually be possible. The potential also exists for the development of so-called "conditional suicide genes," which could program an organism to die off following a predetermined number of replications in the environment. Thus, an affected area may be safely reoccupied after a predetermined period of time.

FOREIGN TECHNOLOGY ASSESSMENT (See Figure 3.0-2)

Seed stocks of the AG group of biological agents are readily available in the natural environment and from culture collections in the industrialized and in some developing nations. The recent outbreaks of Ebola in Africa and Hanta virus infections in Asia and North and South America are evidence of this. In addition, these organisms may be obtained from national collections (e.g., American Type Culture Collection [ATCC] and European collections).

Most industrialized nations manufacture equipment and materials necessary for the production, containment, purification, and quality control of these materials. Canada, France, Germany, Israel, Japan, the Netherlands, Russia, Sweden, Switzerland, the Ukraine, the UK, and the United States are the most advanced countries in the techniques of manufacturing large quantities of biological agents and protective vaccines and materials required for prophylaxis and therapy.

Table 3.1-1. Biological Material Production Technology Parameters

Technology	Sufficient Technology Level	Export Control Reference	Critical Materials	Unique Test, Production, and Inspection Equipment	Unique Software and Parameters
HUMAN PATHOGENS See Figure 3.0-2					
Viruses	Any quantity is a concern. Less than 20 pounds can incapacitate humans in a 10-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Cell culture apparatus; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Bacteria	Any quantity is a concern. Less than 220 pounds can incapacitate humans in a 100-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Fermenters; cell cultures; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Toxins	Any quantity is a concern. Less than 600 pounds can incapacitate humans in a 100-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Fermenters; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Rickettsiae	Any quantity is a concern. Less than 100 pounds can incapacitate humans in a 10-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Cell culture apparatus; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Genetically Modified Microorganisms	Any quantity is a concern.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Infectivity of cultured organisms plus items in four entries above.	Not applicable
ANIMAL PATHOGENS See Figure 3.0-2					
Viruses	Any quantity is a concern. Less than 20 pounds can incapacitate animals in a 10-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Cell culture apparatus; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Bacteria	Any quantity is a concern. Less than 220 pounds can incapacitate animals in a 100-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Fermenters; cell cultures; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Genetically Modified Microorganisms	Any quantity is a concern.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Infectivity of cultured organisms plus items in two entries above	Not applicable

Table 3.1-1. Biological Material Production Technology Parameters (cont'd)

Technology	Sufficient Technology Level	Export Control Reference	Critical Materials	Unique Test, Production, and Inspection Equipment	Unique Software and Parameters
PLANT PATHOGENS See Figure 3.0-2					
Viruses	Any quantity is a concern. Less than 30 pounds can affect plants in a 10-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Cell culture apparatus; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Bacteria	Any quantity is a concern. Less than 30 pounds can affect plants in a 10-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Fermenters; cell cultures; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Fungi	Any quantity is a concern. Less than 50 pounds can affect plants in a 10-km ² area.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Fermenters; cell cultures; laminar flow facilities; containment equipment; biological agent detectors	Not applicable
Genetically Modified Microorganisms	Any quantity is a concern.	AG List; WA ML 7; CCL Cat 1C; USML XIV	Not applicable	Infectivity of cultured organisms plus items in three entries above.	Not applicable
EQUIPMENT					
Containment Facilities	Equipment having three or more physical barriers between the agent and the employee.	AG List; CCL Cat 2B	HEPA filters	Toxic agent detectors	Not applicable
Fermenters	Having: a capacity > 100 liters; multiple sealing joints; capable of <i>in situ</i> sterilization in a closed state.	AG List; CCL Cat 2B	Stainless steel; titanium; glass	Toxic agent detectors	Not applicable
Centrifugal Separators	Capable of processing 5-liter batches	AG List; CCL Cat 2B	Smooth surface; Aerosol containment	Toxic agent detectors	Not applicable
Cross-flow Filtration Equipment	Capable of processing 20-liter batches	AG List; CCL Cat 2B	Smooth surface; Aerosol containment	Toxic agent detectors	Not applicable

Table 3.1-2. Biological Material Production Reference Data

Technology	Technical Issues	Military Applications	Alternative Technologies
HUMAN PATHOGENS See Figure 3.0-2			
Viruses	Containment and dissemination	Biological agents in biological weapons	Not applicable
Bacteria	Containment and dissemination	Biological agents in biological weapons	Not applicable
Toxins	Containment and dissemination	Biological agents in biological weapons	Not applicable
Rickettsiae	Containment and dissemination	Biological agents in biological weapons	Not applicable
Genetically Modified Micro-organisms	Containment and dissemination	Biological agents in biological weapons	Not applicable
ANIMAL PATHOGENS See Figure 3.0-2			
Viruses	Containment and dissemination	Biological agents in biological weapons	Not applicable
Bacteria	Containment and dissemination	Biological agents in biological weapons	Not applicable
Genetically Modified Micro-organisms	Containment and dissemination	Biological agents in biological weapons	Not applicable
PLANT PATHOGENS See Figure 3.0-2			
Bacteria	Containment and dissemination	Biological agents in biological weapons	Not applicable
Fungi	Containment and dissemination	Biological agents in biological weapons	Not applicable
Genetically Modified Micro-organisms	Containment and dissemination	Biological agents in biological weapons	Not applicable

Note: The United States has forsworn the use of biological weapons; however, to perfect defensive procedures, it is necessary to understand the organisms.

(cont'd)

Table 3.1-2. Biological Material Production Reference Data (cont'd)

EQUIPMENT			
Containment Facilities	Protection of the environment and the employee.	Containment, isolation, and production of biological agents	Programs to automate process, allowing automatic control, control from remote locations, and automatic data logging
Fermenters	Cleanliness of facilities and contamination of the agent	Containment, isolation, and production of biological agents	Programs to automate process, allowing automatic control, control from remote locations, and automatic data logging
Centrifugal Separators	Cleanliness of facilities and contamination of the agent	Containment, isolation, and production of biological agents	Programs to automate process, allowing automatic control, control from remote locations, and automatic data logging
Cross-flow Filtration Equipment	Quality of the filters and amount of air-flow	Containment, isolation, and production of biological agents	None identified

Note: The United States has forsworn the use of biological weapons; however, to perfect defensive procedures and intelligence-gathering procedures, it is necessary to understand the manufacturing procedures for these organisms.

SECTION 3.2—STABILIZATION, DISSEMINATION, AND DISPERSION

OVERVIEW

Biological weapons production can be divided into three distinct phases: biological agent production (see Section 3.1), stabilization, and dissemination/dispersion. This section discusses the latter two parts. Stabilization and dissemination/dispersion are important issues because of the susceptibility of the biological agents to environmental degradation, not only in storage but also in application. This is a problem whether the end use is for biological weapons, pharmaceuticals, cosmetics, pesticides, or food-related purposes and is related to the susceptibility of the organisms to inactivation of the biochemical compound by the environment. This loss of bioactivity can result from exposure to high physical and chemical stress environments, such as high surface area at air-water interfaces (frothing), extreme temperatures or pressures, high salt concentrations, dilution, or exposure to specific inactivating agents.

This section discusses various techniques of stabilization, such as freeze drying and ultra freezing, and various techniques of dissemination/dispersion, such as spray devices, cluster bombs, etc. Section 1 of this document discusses modes of delivery, such as cruise missiles, airplanes, and artillery shells .

The primary means of stabilization for storage or packaging are initial concentration; direct freeze drying (lyophilization); direct spray drying; formulation into a special stabilizing solid, liquid, or sometimes gaseous solution; and deep freezing. Methods of concentration include vacuum filtration, ultrafiltration, precipitation, and centrifugation. Freeze drying is the preferred method for long-term storage of bacterial cultures because freeze-dried cultures can be easily rehydrated and cultured via conventional means. Many freeze-dried cultures have remained viable for 30 years or more.

Deep or ultra freezing of biological products is another long-term storage technique for species and materials not amenable to freeze drying. The method involves storage of the contained products in liquid nitrogen refrigerators (−196° Celsius) or ultra-low temperature mechanical freezers (−70° Celsius). Mechanical freezing systems should include precautionary back-up freezers and electrical generators. Cryoprotective agents, such as dimethyl sulfoxide (DMSO), glycerol, sucrose, lactose, glucose, mannitol, sorbitol, dextran, polyvinylpyrrolidone, and polyglycol, are required to ensure cell viability during storage. A toxin agent is most effective when prepared as a freeze-dried powder and encapsulated. Such encapsulation, however, is not necessary for weaponization. Infectious biological agents are generally stabilized and then spray dried.

Effective delivery of these agents must also consider the environmental effects on the agent (inactivation). Dissemination (delivery) of biological agents in biological

Highlights

- Stabilization is critical to effective dissemination.
- The environment can affect the survival of the organism.
- Explosive delivery means can result in inactivation of the organism.

warfare has been traditionally accomplished by aerosol dispersal using either spray devices or through incorporation of the agents with *explosive devices* (cluster bombs, missile warheads with submunitions designed for extended biological agent dispersal). The latter, however, must be approached with caution since explosive, heat-generating entities can inactivate the organisms/toxins. The preferred approach is dispersion via the use of a pressurized gas in a submunition. Other preferred platforms from an efficiency standpoint include small rotary-wing vehicles, fixed-wing aircraft fitted with spray tanks, drones, bomblets, cruise missiles, and high-speed missiles with bomblet warheads. Fixed-wing aircraft and ground vehicles with aerosol generators also make excellent delivery systems.

Aerosolization of biological agents using spray devices is the method of choice since the extreme physical conditions associated with explosive dissemination can completely inactivate the biological agent. (Aerosol dispersal allows for control of particle size and density to maximize protection from environmental degradation and uptake of the enclosed biological agents in the lungs of targeted populations.) Aerosol particles with a diameter of 1–15 μm mass median diameter (MMD) are readily absorbed by lung cells following inhalation, the primary mode of infection by most biological agents. Some agents can also act following ingestion of contaminated food or water. However, infectious agents generally do not penetrate intact skin. Equipment used with aerosol dispersal of biological agents includes spray nozzles or aerosol delivery systems capable of dispersing particles or droplets and compressors for initial weaponization by agent integration with compressed gas (air). For subnational or terrorist groups, the biological agents can be dispersed by manual aerosol generators. The availability of vaccines against selected biological agents may render the user immune to the effects of the agent although a sufficient dose of agent may overwhelm the vaccine's protective effect.

Dissemination efficiency rates of aerosol delivery systems are in the range of 40–60 percent. Cruise missiles, aircraft carrying gravity bombs or spray attachments,

and fixed-wing or rotor craft with attached sprayers are all vehicles for delivery of biological agents. The delivery of biological agents by explosive devices is much less efficient (~1–5 percent).

In a theater environment, the effective use of BW agents requires analysis of meteorological conditions and the mapping of the target.

RATIONALE

Biological agents have some unique characteristics that make weaponizing them attractive. Most biological weapons consist of living organisms (toxins are the exception) and, thus, can replicate once disseminated. A relatively small group of persons, using single individuals deployed in a military staging area, could bring about the infection of a large percentage of targeted persons. The clinical illness could develop within a day of dispersal and last for as long as 2–3 weeks. The mission and political impact of such a strike on a combat or constabulary force of 10,000 soldiers may compromise operations. In a civil situation, major subway systems in a densely populated urban area could be targeted for biological agent strike, resulting in massive political and social disorganization. Approximately 10 grams of anthrax spores can kill as many persons as a ton of sarin. Under appropriate meteorological conditions and with an aerosol generator delivering 1–10 micron particle-size droplets, a single aircraft can disperse 100 kg of anthrax over a 300 km² area and theoretically cause

3 million deaths in a population density of 10,000 people per km². The mean lethal inhalator dosage is 10 nanograms.

On the other hand, some biological agent characteristics can severely limit the effectiveness of BW, which consist of living organisms. A technique to stabilize (protect) the organisms from adverse environments is essential if the weapons are to maintain their effectiveness over some period of time. This requirement of stabilization also extends to the methods of delivery since the organisms are very susceptible to degradation in the environments associated with delivery systems.

FOREIGN TECHNOLOGY ASSESSMENT (See Figure 3.0-3)

Any country having pharmaceutical, cosmetic, or advanced food storage industries will have stabilization facilities similar to those that could be used for biological weapons. The ability to disseminate the biological agent over a wide area would be limited to those countries having cruise missiles or advanced aircraft. Even the smallest country or a terrorist group, however, has the capability to deliver small quantities of BW agent to a specific target. Canada, France, Germany, Israel, Japan, the Netherlands, Russia, the UK, and the United States have the most advanced techniques of manufacturing large quantities of biological agent and are also the most apt to have the capability to disseminate the biological agent over large areas.

Table 3.2-1. Stabilization, Dissemination, and Dispersion Technology Parameters

Technology	Sufficient Technology Level	Export Control Reference	Critical Materials	Unique Test, Production, and Inspection Equipment	Unique Software and Parameters
Freeze-drying Equipment	Having: steam sterilizable; a condenser capacity > 25 kg in 24 hours and < 400 kg in 24 hours	AGList; CCL Cat 2B	Stainless steel; titanium; glass	Toxic agent detectors	None identified
Aerosol Inhalation Chambers	Designed for aerosol challenge testing having a capacity > 0.5 cubic meter	AGList; CCL Cat 2B	High efficiency filter that passes particles 0.1 to 10 µm in diameter	Toxic agent detectors	None identified
Delivery systems and spray tanks to allow bomblet dissemination	Any capability is a concern	WA ML 4, 7; USML IV, XIV	None identified	Spin flow and flow-forming machines	None identified
Warheads for missiles	Any capability is a concern	WA ML 4; USML IV, XIV	None identified	Spin flow and flow-forming machines	None identified
Development and use of accurate, short-term weather prediction	Any capability is a concern	CCL EAR 99	None identified	None identified	Validated software to predict short-term weather patterns

Table 3.2-2. Stabilization, Dissemination, and Dispersion Reference Data

Technology	Technical Issues	Military Applications	Alternative Technologies
Freeze-drying Equipment	Maintaining low temperature	Stabilize biological agents for use in BW or for storage	None identified
Aerosol Inhalation Chambers	Filters that pass 0.1–10 µm particles and remove large quantities of debris (>20 µm diameter)	Testing aerosols for BW use	Detonation-induced release of particles having uncontrolled sizes
Delivery systems and spray tanks to allow bomblet dissemination	Delivery range, accuracy, and effect on contained organisms	Delivery of both conventional weapons and WMD	Detonation-induced release of particles having uncontrolled sizes
Warheads for missiles	Delivery range, accuracy, and effect on contained organisms	Delivery of both conventional weapons and WMD	Balloon-floated devices; non-fixed-wing vehicles
Development and use of accurate, short-term weather prediction	Dissemination of biological weapon	Predict dispersion patterns of disseminated biological weapons to maximize the effect on hostile troops and, at the same time, minimize the effect on friendly troops	On-site determination of wind pattern and wind flow

SECTION 3.3—DETECTION, WARNING, AND IDENTIFICATION

OVERVIEW

Detection, warning, and identification involve sensors and transduction of a detected signal to a transponder. Standoff detectors provide early, wide-area spectroscopy and warning of biological agent attack. Stand-off detectors are spectroscopy-based monitors of materials containing nucleic acid/protein with absorbance in the 230–285 nanometer range. They can be confounded by biological material or pollen of size similar to that of the biological agent. Point detectors are used at designated locations. Most detection and warning systems are based on physical or chemical properties of biological agents. The point detectors include dipstick kits selective for some but not all AG agents (see Table 3.0-2) or multiarray sensors using antibodies generated against AG agents or gene sequences complementary to AG agents. Identification systems, which are critical to medical response, use immunochemical or gene probe techniques or mass spectral analysis. No single sensor detects all agents of interest. Detectors for biological agents must have a short response time (less than 30 minutes for biological agents) with a low false alarm rate. Detection equipment must be integrated with a command and control system to ensure an alarm is raised. Early warning is essential to avoid contamination. Agent location, intensity, and duration are crucial parameters for command decisions.

Sensor systems based on physical or chemical properties of biological agents include high-performance liquid and gas chromatography, mass spectrometry, scattering Light Detection and Ranging (LIDAR), and ion mobility spectrometry (IMS). The basic recognition component of the sensor designed for a specific agent is generally a large molecule that binds selectively to the target agent. The recognition molecules are physically bound to a supporting surface that generates a signal (transduction) when the recognition molecule binds the biological agent. The methods for transduction include (1) changes in absorption of light at specific wavelengths; (2) changes in resonating frequency of a piezoelectrically active surface caused by mass effects; (3) changes in pathways of light movement at an interface of target agent and recognition molecules; and (4) switching of a light-conducting pathway resulting from interaction of recognition molecule with the biological agent. Recognition molecules are antibodies (association constants of 10^{-6} to 10^{-8}), receptors (dissociation constant, KD, $\text{KD} = <10^{-14}$), or DNA sequences complementary to genetic material encoded by the biological agent.

Highlights

- Reliable, quick-response sensor systems are essential for detection and warning.
- Identification is critical to medical response.
- Various physical phenomena are used to convert sensor signals to useful detection and identification information.
- Underlying sensor technology exists in many countries.

Biodetection systems providing limited warning and identification functions currently exist. Systems in the inventory or in the advanced stages of development warn that a biological attack has occurred and collect samples for subsequent laboratory analysis. However, no real-time, on-site detection systems are available today. The rapid growth in biotechnology is assisting in the area of improved biological defense technologies, although many of the same advances can also be used to improve biological agents.

RATIONALE

Early detection and warning is the first line of defense against biological agents. Detection and identification of biological agents allow commanders to take steps to avoid contamination, to determine the appropriate protection for continued operations, and to initiate proper prophylaxis and therapy to minimize casualties and performance degradation.

FOREIGN TECHNOLOGY ASSESSMENT (See Figure 3.0-3)

Besides the United States, several countries have a significant capability in the sensor technology that underlies detection and identification of biological agents: Canada, France, Germany, Israel, Japan, The Netherlands, Russia, Sweden, and the UK. Several other countries are just a step behind: Austria, China, Czech Republic, Finland, Hungary, Slovak Republic, South Africa, Switzerland, and the Ukraine. The worldwide efforts to develop improved biological agent detectors are extensive.

Table 3.3-1. Detection, Warning, and Identification Technology Parameters

Technology	Sufficient Technology Level	Export Control Reference	Critical Materials	Unique Test, Production, and Inspection Equipment	Unique Software and Parameters
Immuno-based detectors	Capability of detecting organisms of AG agents	WA ML 7; WA IL Cat 1A; USML XIV	Antibodies directed against AG list agents	Antibody development	None identified
Gene-based probe	Capability of detecting organisms of AG agents	WA ML 7; WA IL Cat 1A; USML XIV	Polynucleotides complementary to AG gene sequences; polymers	Gene sequence data	None identified
Molecular recognition (e.g., antigens, antibodies, enzymes, nucleic acids, oligomers, lectins, whole cells, receptors, organelles)	Capability of detecting organisms of AG agents. Can recognize weapons grade agent, by-products of its preparation or manufacturing signatures; does not recognize normally occurring environmental materials.	WA ML 7; WA IL Cat 1A; USML XIV	Antibodies directed against AG List agents or polynucleotides complementary to AG gene sequence	Coatings, films, or fibers of biopolymers or chemical polymers that bind BW agents (binding Kd less than 1×10^{-8})	Molecular modeling (e.g., protein and DNA sequencing)
Mass Spectrometry	Capable of scanning samples of 10,000 daltons or less in 30 minutes or less	WA ML 7; WA IL Cat 1A; USML XIV	None identified	Database development; portable, field-rugged mass spectroscopy	Spectrum recognition algorithms
IMS	Detecting hundreds of organisms	WA ML 7; WA IL Cat 1A; USML XIV; CCL Cat 6	None identified	Database development; ion source; spectroscopy capable of concentrating and analyzing 1,000 organisms	Spectrum recognition algorithms
Scattering LIDAR	Detect agent (liquids and aerosols) at any distance	WA ML 7; WA IL Cat 1A; USML XIV	None identified	None identified	Spectrum and background recognition algorithms
Transducers [e.g., optical, electrochemical, acoustic, piezoelectric, calorimetric, Surface Acoustic Wave (SAW); fiber-optic wave guide]	Converts recognition of agents to an optical or electrical signal; low hysteresis; optical/electronic component processing within 30 minutes	WA ML 7; WA Cat 3A; USML XIV; CCL Cat 3A	None identified	Production equipment configured for the detection of biological agents	Spectrum recognition algorithms

(cont'd)

Table 3.3-1. Detection, Warning, and Identification Technology Parameters (cont'd)

Technology	Sufficient Technology Level	Export Control Reference	Critical Materials	Unique Test, Production, and Inspection Equipment	Unique Software and Parameters
Sample Collection (e.g., air, liquid, dust, soil sampling)	Collects and concentrates <10 µm particles into liquid medium	WA ML 7; USML XIV	None identified	Aerosol samplers able to collect ≤10 µm diameter particles into a liquid	None identified
Sample Processing (e.g., cell disruption, concentration, purification, or stabilization)	Completion within 30 minutes	WA ML 7; USML XIV	None identified	Neg. pressure orifice devices for rupturing cell membranes or wall/retention of nucleic acids; impact collectors; ion trap mass spectrometers capable of scanning samples below 10,000 daltons in 5 minutes or less; pyrolyzers	Spectrum recognition algorithm
Development and use of sensor models	Specific performance of military sensors	USML XIII	Software/technical data for military systems on control lists	None identified	None identified

Table 3.3-2. Detection, Warning, and Identification Reference Data

Technology	Technical Issues	Military Applications	Alternative Technologies
Immuno-based detectors	Low cross-reaction of antibodies with non-pathogenic organisms	Confirmation and All Clear device; screening device	Light scattering (e.g., LIDAR) not specific for agent; culture and morphological characterization of the agent
Gene-based probe	Obtaining the sufficient length of nucleic acid sequence (approx. 30 to 40 polynucleotides) to define the pathogen	Characterization and identification of AG agents; enables the conversion of pathogenic to non-pathogenic organisms and vice-versa	Light scattering (e.g., LIDAR) not specific for agent; culture and morphological characterization of the agent
Molecular recognition (e.g., antigens, antibodies, enzymes, nucleic acids, oligomers, lectins, whole cells, receptors, organelles)	Identifying specific epitopes or genetic sequences characteristic of threat agents; designing probes that are specific for the epitopes or sequences that are stable under the conditions of use and can be incorporated into the sensor	Contamination avoidance; biological agent detection; process and quality control in biological agent manufacturing	Light scattering (e.g., LIDAR) not specific for agent; culture and morphological characterization of the agent
Mass Spectrometry	Requires sophisticated software; must know what you are looking for; extremely powerful analytical tool; training/maintenance requirements higher; requires significant power; size and weight problems	Identification of agents	Stand-off technologies including light scattering (e.g., LIDAR) not specific for agent; culture and morphological characterization of the agent
IMS	Detect broad range of biological materials, including agents; short response time; semi-quantitative	Alarm with potential for individual application, monitoring; early warning	Immuno-based detectors, gene-based probes, and molecular recognition; culture and morphological characterization of the agent
Scattering LIDAR	Background varies widely; size, power and weight requirements; need frequency agile laser	Early interrogation of suspect aerosol clouds	Immuno-based detectors, gene-based probes, and molecular recognition; culture and morphological characterization of the agent
Transducers (e.g., optical, electrochemical, acoustic, piezoelectric, calorimetric, SAW; fiber optical wave guide)	Miniaturization, stability to environment and exposure to samples; reproducibility, calibration; simplicity of use	Contamination avoidance; biological, chemical agent detection	Culture and morphological characterization of the agent
Sample Collection (e.g., air, liquid, dust, soil sampling)	100–1,000 liters of air per minute; sample preparation; separation and concentration of biological agent	Contamination avoidance; biological agent detection; process and quality control.	Appearance of illness in exposed personnel
Sample Processing (e.g., cell disruption, concentration, purification, or stabilization)	Sample processing while maintaining integrity of agent; automation and miniaturization; amplification techniques	Contamination avoidance; biological agent detection; process and quality control in biological/toxin agent manufacturing.	Appearance of illness in exposed personnel
Development and use of sensor models	Clutter characteristics; specific sensor techniques for clutter rejection/sub-clutter target detection/identification	C3I; mission rehearsal	Appearance of illness in exposed personnel

SECTION 3.4—BIOLOGICAL DEFENSE SYSTEMS

OVERVIEW

This section covers measures that can be taken to protect forces in a biological weapons environment. The protection and countermeasures issues related to biological warfare and defense concern the individual soldier and the unit.

The individual soldier can be protected by providing prophylactic treatment before deployment into a risk area, by providing full respiratory protection during time periods of potential exposure [Mission-Oriented Protection Posture (MOPP) gear] to the biological agent, or by using pharmacological, physical, or biomedical antidotes to threat agents shortly after exposure. Prophylaxis of the individual is generally accomplished by immunization, using the attenuated or dead biological agent, which serves as an immunogen. More recently, it has become possible to provide protection by immunizing personnel against a fragment of the toxin/biological agent. Initiating the immunization process to achieving protection usually involves a period of weeks. Multivalent vaccines and DNA vaccines are in development to enhance countermeasures against biological agents.

Protection measures for a unit or group primarily rely on weather monitoring, remote probe monitoring for biological agents, and central command data acquisition, transfer, and analysis. Large-scale decontamination measures for barracks, vehicles, and other equipment are also considered unit protection.

Individuals can be protected from exposure to biological weapons agents by active or passive immunization against the agents. Figure 3.0-2 has identified many of the agents of concern. A nation's capability to use a biological agent should be limited by its ability to provide protection against the agent for its forces and civilian population. A proliferant may not recognize such a limit. In addition, administering biological response modifiers (BRMs) to personnel at the appropriate time can mobilize the immune system in a normal individual. This will reduce the likelihood that exposure to a biological or toxin agent will degrade the individual's function or result in disease or death. These performance enhancers (BRMs) are discussed in detail below.

BRMs or immunomodulators are biomolecules with the ability to enhance or diminish the immune response of the body. During the last decade, several BRMs (e.g., interferons, interleukins) have been identified. When injected, they enhance the immune response of the human subject to a given antigen (virus or bacterium). Derivatives of these immune enhancing agents can be administered to personnel to improve performance efficiency.

Several naturally occurring proteins, including interferons and interleukins, function as immunostimulating BRMs. In addition to naturally occurring BRMs such as

Highlights

- A proliferant would require some type of BW defensive capability for protection during employment and defense against a counter-attack.
- Vaccines are possible but the agent must be known (requires lead time for full protection).
- Detection and identification are key to determine appropriate defensive measures to take after an attack.
- A mask is sufficient to prevent a majority of biological agents from infecting personnel.
- Biotechnology offers potential for enhanced protection in the future.

interferons and interleukins, immuno-enhancing drugs, such as arspenamamine and cefodizime, act to stimulate natural immune response. These drugs are used widely in medicine following chemotherapy and for treatment of various autoimmune diseases. Growth factors for cells of the hematopoietic immune system have been found useful for ameliorating immunosuppression conditions. BRMs can be administered via conventional methods, using encapsulation technology for mass treatment through aerosols or using controlled release systems for long-term internal treatment. Although the immune system enhancers are of potential benefit, they may have undesired side effects, such as fever and malaise, that can degrade combat performance.

Anti-idiotype antibodies can be used to initiate immunization in forces against toxic biological agents. Immunization with the anti-idiotype can induce production of antibodies that recognize and bind the biological agent specifically and selectively. In the most favorable scenario, the human subject would be completely protected immunologically and yet never be exposed to attenuated biological or toxin agent.

Immunosuppressants are one class of BRMs that show promise in offensive biological warfare. These are substances that cause subjects to become "immuno-compromised" or more susceptible to infection and, therefore, can be used directly or in concert with other encapsulated chemical or biochemical weapons for diminishing an adversary's capabilities. These substances include pharmaceuticals, such as

cyclosporin, rapamycin, and FK506, which are useful in chemotherapy treatments for various cancers and in the prevention of organ, bone marrow, or skin graft rejection.

Biological agent protection requires only respiratory and eye protection rather than the complete MOPP gear required for chemical protection. The protective garment requirements include resistance to the penetration of biological weapon or toxin materials, filtration of inflow air to remove particles containing the agents, and cooling of the interior compartment.

Current clothing and mask systems used for protection against biological agents act as a barrier between the agent and the respiratory system or mucosal tissues of the target. They do not inactivate the agent. For biological protection, such clothing is sufficient but is not comfortable. Visual field of view is decreased and the head mask results in discomfort because of temperature increase and fogging.

RATIONALE

Biological defense systems technologies have been included for two reasons. First, an aggressor can be expected to have some standard of protection for the force employing BW. Standards of protection could vary from minimal to sophisticated, but all should be considered, especially those that allow a proliferant to feel secure in

offensive operations. Secondly, an attacker would have to be prepared for a counter-attack in kind (depending on the opponent).

Self-protection defensive measures would be easiest to take in an offensive attack mode. The attacker would know in advance what biological weapon(s) would be employed and could immunize those that might come in contact with the organism(s). Protective masks could be worn to provide additional protection.

When being attacked, a country would encounter problems similar to those faced by the United States: unknown agents being used at an unspecified place for an undetermined duration. Immunization requirements would have to be determined by intelligence reports of enemy capabilities. Some type of detection (see Section 3.3) would be needed to alert forces to take protective measures.

FOREIGN TECHNOLOGY ASSESSMENT (See Figure 3.0-3)

Vaccines can be produced by any country with a pharmaceutical industry. Equipment can be purchased on the open market since it is all dual use. Protective masks are made in many countries. A simple dust mask could provide significant protection as long as it was worn when being exposed to the biological agent.

Table 3.4-1. Biological Defense Systems Technology Parameters

Technology	Sufficient Technology Level	Export Control Reference	Critical Materials	Unique Test, Production, and Inspection Equipment	Unique Software and Parameters
Production and design technology for protective masks	Any capability	WA ML 7; WA Cat 1A; USML X	Butyl rubber; silicone rubber	Simulated agents; leakage testers; mannequin-face model for mask and suit design; particle-size analysis equipment	Software for generating facial contours
Production and design technology for collective protection	Any capability	WA ML 7; USML X	Teflon/Kevlar laminate for biological resistance, decontaminability and environmental durability	Simulated agents	None identified
Decontamination	Any capability	WA ML 7; USML XIV	Hypochlorite or similar bleach compound or autoclaving for sterility	None identified	None identified
Vaccines	Any capability	CCL EAR 99	Target strains	None identified	None identified
BRMs	Any capability	CCL EAR 99	None identified	None identified	None identified
Regenerative collective protection - Membrane filtration	Any capability	WA ML 7; USML XIV	Filter system to remove 0.1- to 15-micron particles by sieve action	Simulated agents; particle-size analysis equipment	None identified
Regenerative collective protection - Plasma destruction	Any capability	WA ML 7; USML XIV	Portable plasma generator	Simulated agents; recovery of infectious agent	None identified
Encapsulation: liposomes; polymer entrapment; micelles; emulsions; immobilization of biopolymers	Any capability	CCL EAR 99	None identified	None identified	None identified
Antibiotics	Any capability	CCL EAR 99	None identified	None identified	None identified

Table 3.4-2. Biological Defense Systems Reference Data

Technology	Technical Issues	Military Applications	Alternative Technologies
Production and design technology for protective masks	Communications (microphone pass-through); respiration (air management); eye protection; composite eye lens retention system; anthropometrics; performance degradation; ability to consume fluids	Protective masks that are suitable in removing aerosol dispersed biological agents	Avoid contamination
Production and design technology for collective protection	Affordable; deployable; adaptable to structure	Continue to operate without degradation	Individual protection
Decontamination	Volume of agent; time required; adaptability to unknown agents; environmentally sound; identification of what needs to be decontaminated; identification of decrease of toxicity to allowable level	Reduce contamination to allow military operations	Oxidizing or chlorinating chemical treatment; heat at 120 °C with pressure
Vaccines	Efficacy of vaccine; efficacy of prophylaxis; pre- vs. post-exposure treatment	Minimize BW casualties; reconstitute forces; maintain performance standards	Preclude viral or bacterial entry or maturation in target tissue
BRMS	Efficacy of prophylaxis; pre- vs. post-exposure treatment	Minimize casualties after BW attack; reconstitute forces; maintain performance standards	Enhance immune response
Regenerative collective protection - Membrane filtration	Remove particles having average diameter of 0.1–15 µm, and allow rapid flow of air	Reduction of logistics burden; preclude inhalation of aerosolized biological agent	Standard filters
Regenerative collective protection - Plasma destruction	Production of lightweight plasma generators (e.g., ozone that is bactericidal or inactivates viruses)	Reduction of logistics burden; inactivate aerosolized biological agent	Standard filters
Encapsulation; liposomes; polymer entrapment; micelles; emulsions; immobilization of biopolymers	Ensure release of prophylaxis and therapeutics shortly after contact with plant/animal/human tissues	Individual protection; decontamination; performance retention	None identified
Antibiotics	Inhibit cysteine proteases or cellular transport	Minimize casualties after BW attack; reconstitute forces; maintain performance standards	Preclude viral or bacterial entry or maturation in target tissue