

# BIOLOGICAL WEAPONS—A PRIMER FOR MICROBIOLOGISTS<sup>1</sup>

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■ **Abstract** Biological weapons are not new. Biological agents have been used as instruments of warfare and terror for thousands of years to produce fear and harm in humans, animals, and plants. Because they are invisible, silent, odorless, and tasteless, biological agents may be used as an ultimate weapon—easy to disperse and inexpensive to produce. Individuals in a laboratory or research environment can be protected against potentially hazardous biological agents by using engineering controls, good laboratory and microbiological techniques, personal protective equipment, decontamination procedures, and common sense. In the field or during a response to an incident, only personal protective measures, equipment, and decontamination procedures may be available. In either scenario, an immediate evaluation of the situation is foremost, applying risk management procedures to control the risks affecting health, safety, and the environment. The microbiologist and biological safety professional can provide a practical assessment of the biological weapons incident to responsible officials in order to help address microbiological and safety issues, minimize fear and concerns of those responding to the incident, and help manage individuals potentially exposed to a threat agent.

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## INTRODUCTION

Biological weapons derived from biological material(s) are considered weapons of mass destruction or, more appropriately, as weapons of mass casualty. A biological weapon can be considered a four-component system composed of a payload, munition, delivery system, and dispersion system (20). The payload is biological material consisting of an infectious agent (a pathogen) or a toxin produced by bacteria, plants, or animals. The munition serves to containerize the payload to maintain its potency during delivery. The delivery system can be a missile, vehicle (aircraft, boat, automobile, or truck), or an artillery shell to transport the payload to a susceptible target. The dispersion system, provided by an explosive force or spray mechanism, ensures dissemination of the payload at the intended target.

Although the list of potential agents is numerous, those agents that could cause mass casualties by the aerosol route of exposure are considerably smaller (8, 10–12, 15, 21, 27, 41, 57, 80). Infectious biological payloads that could potentially be used include those causing anthrax (*Bacillus anthracis*), plague (*Yersinia pestis*), tularemia (*Francisella tularensis*), equine encephalitides (Venezuelan equine encephalitis, eastern equine encephalitis, and western equine encephalitis viruses), hemorrhagic fevers (arenaviruses, filoviruses, flaviviruses, and bunyaviruses), and smallpox (variola virus). Toxins include botulinum toxin from *Clostridium botulinum*; ricin toxin from the castor bean *Ricinus communis*; trichothecene mycotoxins from *Fusarium*, *Myrothecium*, *Trichoderma*, *Stachybotrys*, and other filamentous fungi; staphylococcal enterotoxins from *Staphylococcus aureus*; and toxins from marine organisms such as dinoflagellates, shellfish, and blue-green algae. If these agents are delivered successfully to a susceptible host, a lethal or incapacitating outcome will occur, depending upon the agent. For instance, the agents causing anthrax and plague will most likely result in death of the target host. *Coxiella burnetii* (the agent of Q-fever), staphylococcal enterotoxin B, and Venezuelan equine encephalitis virus are considered incapacitating agents. In a military context, incapacitating agents may be in a certain sense more effective because the unit will not be able to perform their mission and casualties will consume scarce medical and evacuation assets.

Both lethal and incapacitating agents could have an adverse impact on the civilian healthcare delivery system in a biological terrorism scenario. Potential manifestations include terror in the affected population and in medical care personnel; an overwhelming number of casualties placing demands for ICU care or special medications; a need for personal protection in medical care settings, clinical laboratories, and autopsy suites; and problems with handling remains. Employing standard precautions or barrier nursing techniques (23), depending on the agent, can provide appropriate (or adequate) protection to healthcare providers against biological agents. However, additional precautions such as aerosol, droplet, or contact protection are recommended in instances where smallpox, *Y. pestis* (plague), the hemorrhagic fever viruses, or T-2 mycotoxin are suspected. There may be a

hazard of person-to-person transmission, transmission by direct contact with blood or body fluids, or dermal activity (13, 27, 28, 31, 32, 65, 86).

## HISTORICAL ASPECTS

Biological weapons are not new, but the technologies of production and delivery have been developed and perfected by nations during the twentieth century. For thousands of years, biological agents have been available as instruments of warfare and terror (bioterrorism) to produce fear and harm in a vulnerable population. In addition to the general human population as possible targets, animals and plants have also been possible targets for those desiring to use these agents. To initiate an epidemic of plague during the fourteenth century siege of Kaffa, the attacking Tatar force catapulted the bodies of their deceased soldiers into the city. During the 1754–1767 French and Indian War, the British contemplated the deliberate use of smallpox against Native American tribes. An opportunity to exercise the plan using fomites occurred during an outbreak of smallpox at Fort Pitt. On June 24, 1763, blankets and a handkerchief were given to the immunologically naïve Native Americans in an attempt to “reduce” tribes hostile to the British (10).

Germany developed plans during World War I to contaminate animal feed and infect livestock for export to Allied forces. They also planned to infect sheep from Romania with *B. anthracis* (anthrax) and *Burkholderia mallei* (glanders) for export to Russia. German saboteurs operating in Mesopotamia allegedly were to use *B. mallei* to inoculate 4500 mules and infect horses of the French cavalry in France (17). Livestock in Argentina, destined for export to Allied forces during 1917–1918, were infected with *B. anthracis* and *B. mallei*, resulting in the death of more than 200 mules (10).

Japan conducted biological warfare experiments in Manchuria from 1932 to 1945 (88). At the infamous Unit 731, a biological warfare research facility near the town of Ping Fan, prisoners were infected with *B. anthracis*, *Neisseria meningitidis*, *Shigella* spp., *B. mallei*, *Salmonella typhosa*, *Vibrio cholerae*, *Y. pestis*, smallpox virus, and other disease-causing agents (34). In addition, a number of Chinese cities were attacked with biological warfare agents. The Japanese contaminated water supplies and food items with *B. anthracis*, *Shigella* spp., *Salmonella* spp., *V. cholerae*, and *Y. pestis*. Cultures were also tossed into homes and sprayed from aircraft. Potentially infected fleas were harvested in the laboratory and as many as 15 million fleas were released from aircraft per attack. Because of inadequate preparation, training, and/or lack of proper equipment by the Japanese, the Chekiang Campaign in 1942 reportedly led to about 10,000 biological casualties and 1,700 deaths among the Japanese troops, most from cholera and some from dysentery and plague (89).

Prisoners in Nazi concentration camps were forcibly infected with *Rickettsia prowazekii*, *Rickettsia mooseri*, hepatitis A virus, and *Plasmodium* spp., and also treated with investigational vaccines and drugs (48). The Germans polluted a

large reservoir in northwestern Bohemia with sewage in May 1945 (76), the only known tactical use of biological warfare by the Germans. For potential retaliatory use in response to a German biological attack, the British developed biological warfare capabilities by conducting bomb experiments with weaponized spores of *B. anthracis* on Gruinard Island near the coast of Scotland during 1941 and 1942. Viable anthrax spores persisted for 45 years after World War II until the island was decontaminated with formaldehyde and seawater in 1986 (44).

The United States' offensive biological warfare program began in April 1943 at Camp Detrick, Maryland (renamed Fort Detrick in 1956), with testing sites at Horn Island, Mississippi, and Granite Peak, Utah (22). Experiments were conducted with *B. anthracis* and *Brucella suis*. A new production facility was constructed at Pine Bluff, Arkansas (45), during the Korean War (1950–1953) to meet projected demands. Human experiments were conducted at Camp Detrick with non-lethal agents in 1955. Exposure of volunteers to biological munitions containing *F. tularensis* and *C. burnetii* occurred in a 1-million-liter spherical aerosolization chamber. Volunteers put their face up to an opening in a portal for dosage studies. Studies were designed and conducted to determine the volunteers' vulnerability to aerosolized pathogens and the efficacy of vaccines, prophylaxis, and therapies under development. Additional studies were done with the simulants *Aspergillus fumigatus*, *B. subtilis* var. *globigii*, and *Serratia marcescens* to determine production and storage techniques, aerosolization methods, the behavior of aerosols over large geographic areas, and the effects of solar irradiation and climatic conditions on the viability of aerosolized organisms.

During 1952–1953, Moscow, Peking, and Pyongyang alleged that US Armed Forces used biological weapons against targets in North Korea and China. Although these allegations were not conclusively proven true or false by the available evidence, and were denied by the US Government, they caused the United States a loss of international good will. Belief in the US use of biological weapons during this period of time still persists. The events in Brazil during 1957–1963 are reminiscent of the technique used by the British against Native American Indians in the eighteenth century. In the 1969 trial, the Brazilian Ministry of the Interior disclosed evidence on the deliberate use of the agents smallpox, chickenpox, tuberculosis, influenza, and measles on several Indian tribes in the Mato Grosso. These agents were allegedly introduced in order to clear the Indian tribes from valuable rubber land. The *South China Morning Post* reported that Chinese officials accused the United States of plotting the cholera epidemic in the southeast province of Kwantung Province in the summer of 1961. The US Department of State denied this accusation (77).

During the 1962–1968 war in Indo-China, Viet Cong rebels used a crude form of biological weapon. They used spear traps and hidden bamboo spikes (“pungi sticks”) tipped with animal or human excrement as a booby trap hazard for US forces in Vietnam. These weapons produced casualties by causing percutaneous infection in victims after they came in contact with the spear traps and spikes (18, 78). US allegations of the use of aerosolized trichothecene mycotoxins (“yellow

rain”) in Laos (1975–1981), Kampuchea (1979–1981), and Afghanistan (1979–1981) by Soviet armed forces and their surrogates is unsubstantiated for many reasons (10). An interesting point must be raised regarding the allegations of biological weapons use by various factions or governments. Conclusive evidence is less likely to be found the longer the time period between a report of the alleged use of a biological weapon and an investigation. Whatever the belief, there is some uncertainty of their use (77).

An attempted assassination in 1978 of the Bulgarian exile Vladimir Kostov in Paris, France used ricin (the toxin extracted from castor beans). A ricin-containing pellet was discharged from an umbrella gun into his back, but because of the heavy clothing he was wearing, the pellet did not penetrate deep enough in his body for the wax pellet coat to melt. The Bulgarian exile Georgi Markov was assassinated 10 days later in London with a ricin-filled polycarbonate ball (46). An umbrella gun discharged the pellet into the subcutaneous tissue of his leg while he was waiting at a metro stop. Despite care administered during his hospitalization, he died three days later (19).

Use, or the potential use, of biological weapons during the past 20 years has been exhaustively publicized, especially since the discovery of an extensive biological warfare program in Iraq after the Gulf War of 1991. Coupled with knowledge of the covert program in the former Soviet Union (2, 3), considerable public attention in the United States has been focused on the use and consequences of a biological weapons encounter. The Rajneeshee Cult, an Indian religious group, contaminated restaurant salad bars in Oregon in 1984 with *Salmonella typhimurium*, and about 751 citizens were infected. The cult’s motivation was to incapacitate voters in order to win a local election and to seize political control of Dalles and Wasco counties (74, 85). In 1991, the Minnesota Patriots Council, a group consisting of anti-government tax protesters, planned to inoculate Internal Revenue Service officials, a US Deputy Marshal, and local law enforcement officials through the skin with ricin. Their objective was to cause harm to the federal government and obtain personal revenge (85). Larry Wayne Harris wanted to alert Americans to the Iraqi biological warfare threat and sought a separate homeland for whites in the United States. He had links to Christian Identity and the Aryan Nation, a white supremacist group. Harris made vague threats against US federal officials on behalf of right-wing “patriot” groups. He obtained the *B. anthracis* vaccine strain, *Y. pestis*, and reportedly several other bacteria and discussed the dissemination of biological warfare agents by means of crop duster aircraft and other methods. Harris was arrested in 1998 after he made threatening remarks to US officials and openly talked about biological warfare terrorism (85).

Beyond the shores of the United States, the Aum Shinrikyo (Aum Supreme Truth) Cult sought to establish a theocratic state in Japan with a charismatic, power hungry leader named Shoko Asahara (75). Their objective was to prove an apocalyptic prophecy, eliminate enemies and rivals, halt an adverse court ruling regarding a real estate dispute in Matsumoto in 1994, and seize control of the Japanese government. In 1995, they disseminated the chemical agent sarin

(inhibits acetylcholinesterase, thereby disrupting nerve impulse transmission) in the Tokyo subway system. Their multiple attacks with many chemical agents such as hydrogen cyanide (prevents the normal utilization of oxygen) and VX (mechanism of action similar to that of sarin), and assassinations in other areas of Japan resulted in the injury of more than 1000 persons and the deaths of at least 20 (85). These chemical agents can be incapacitating or lethal, depending upon the dosage. Targets of the Aum Shinrikyo Cult were mass civilian populations, individuals opposed to their ideology, and judges ruling against and police investigating the cult. They attempted to obtain Ebola virus from Zaire, Africa, in 1993, and in 1994 discussed the possibility of using Ebola virus as a biological weapon. The cult also cultured and experimented with the agents of anthrax, Q-fever, cholera, and botulinum toxin (54, 85).

## PROPERTIES OF BIOLOGICAL AGENTS

Biological agents may be used, perhaps as an ultimate weapon, because of several characteristics valued by the perpetrator. Aerosols of biological agents are invisible, silent, odorless, tasteless, and are relatively easily dispersed without detection. They are relatively inexpensive to produce. It is estimated that the cost would be about 0.05% the cost of a conventional weapon to produce a similar amount of mass casualties per square kilometer. In addition, their production follows common fermentation technology that is used for the production of some antibiotics, vaccines, foods, and beverages. Basic, commonly available technology is available for their delivery, such as spray devices from an airplane, boat, or car, over large areas. Terrain, equipment, and infrastructure are usually spared because explosives are not normally used for the delivery of biological agents. Because of this simplicity, it is not difficult for users to tailor their arsenal to fit their needs. Biological weapons can be used in combination with other weapons to create fear, terror, and panic in a population, producing large numbers of casualties. The consequences of their use are many. They may rapidly overwhelm medical resources, and the perpetrators could escape before any effects are even noticed because most agents have incubation times of several hours to days. In addition, the use of an endemic agent may cause confusion because of the inability to differentiate a biological warfare attack from a natural epidemic, and, although limited, a potential exists for secondary or tertiary transmission.

Where can an individual or group obtain biological agents for use in biological warfare or develop biological weapons, and how can these agents be delivered to a susceptible target? Biological agents can be obtained from multiple culture collections, universities, commercial chemical and biological supply houses, foreign laboratories, and from field samples or clinical specimens. Aerosol delivery is considered optimal if the particle size is between 1 and 5  $\mu\text{m}$  because particles with these characteristics will settle in the lower respiratory tract and be undetectable by our senses. Smaller particles will be exhaled because of the aerodynamics of

particle flow through the respiratory tract. Larger particles will settle on environmental surfaces or on the upper respiratory tract, allowing mucocilliary clearance. Delivery of a biological agent by an explosive device is less effective because the heat and light from an explosion may inactivate the agent. Production of particles of 1–5  $\mu\text{m}$  in size is inefficient in an explosion. Delivery methods can include an open-air line source delivery system where a spray device is attached to a moving conveyance. An open-air point source delivery system employs a stationary device such as a sprayer, bombs, or bomblets (a device with a physical guidance system designed to disseminate the biological agent upon impact or at a predetermined altitude above the ground). Limited air-delivery applications include spray devices, bombs, or bomblets. Delivery in water supplies may not be effective because of the dilution factor and because water purification methods such as chlorine treatment, coagulation and flocculation methods, and reverse osmosis systems tend to inactivate microbial agents. There must be increased vigilance, however, in some circumstances because the effectiveness of delivery of a biological agent(s) in water can be increased by its delivery at or near the source of consumption. Biological agents can also be delivered by direct application as in the case of assassination by pellets (46) or flechettes (a dart-shaped projectile) (15).

## Personal Protection

How can we protect ourselves against biological warfare agents? The primary means of protecting both persons working with potentially hazardous biological materials and the environment in a laboratory or research environment may be use of engineering controls, combined with proper good laboratory and microbiological techniques, and common sense. Equally important is the use of certain personal protective equipment and measures (such as an occupational health program) and decontamination procedures. Implementing protective measures and associated procedures depends upon both the situation and their availability. Standard precautions (designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection) and protection against transmission by contact, droplet, and airborne vectors, where appropriate, should be employed by all laboratory workers to prevent their exposure to potentially hazardous materials (37). Standard precautions (29, 30) combine the major features of universal precautions (blood and body fluid precautions designed to reduce the risk of transmission of bloodborne pathogens) and body substance isolation (designed to reduce the risk of transmission of pathogens from moist body substances).

Engineering controls (82) refer to those barriers applied at the point of origin of the hazard. The most important primary barriers are biological safety cabinets, some form of animal cage containment (cage tops with filters and laminar flow enclosures), and positive-pressure protective suits. Biological safety cabinets (e.g., Class I, II, and III cabinets) are the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents (61, 62). Incorporating charcoal filters in the exhaust system of cabinets makes them suitable for use with toxins suspended in volatile material. In situations

where the agent is highly hazardous (and where there are no other protective measures, such as a vaccine against the agent), laboratory workers are physically protected by a positive-pressure protective suit, a type of primary barrier, in biosafety level-4 laboratories.

Secondary barriers of protection against biological agents include design features that protect individuals inside and outside the facility. These barriers vary depending upon the hazards presented by the agents and materials and the laboratory procedures used in the facility (63). Secondary barriers may include physical barriers in the laboratory or research facility (such as limited access, separate room entry areas, personnel airlocks, or change rooms), directional air flow of nonrecirculated air, and discharge of exhaust air remote from occupied areas and air intakes. Exhaust air must be filtered through one or more high-efficiency particulate air (HEPA) filters in series with certain infectious agents or at biosafety level 4. HEPA filters are constructed of paper-thin sheets of borosilicate medium, pleated to increase surface area with aluminum separators for added stability, and affixed to a frame. A HEPA filter removes particulate material the size of  $0.3 \mu\text{m}$  or greater with a minimum efficiency of 99.97% (59, 83). Other forces, such as electrostatic charge and the effects of filtration velocity, impaction, and entrapment, affect filtration efficiency of particles smaller than  $0.3 \mu\text{m}$ . Additional barriers include nonionizing ultraviolet (uv) light irradiation (which is lethal to a wide variety of bacteria and viruses, but its effectiveness depends on the intensity and length of exposure); sinks for handwashing; screened or sealed windows; equipment for decontamination and disposal of hazardous materials; work surfaces amenable to cleaning, housekeeping, and decontamination; and personal protective equipment (PPE).

PPE includes clothing and equipment used to protect individuals in a laboratory or research environment from contact with infectious or toxic materials or physical hazards. The appropriate PPE for an activity depends upon the operations conducted and the potential hazards associated with the activity. It must be emphasized that PPE, although an important item of personal protection, only serves as a secondary barrier against hazards in the laboratory or research environment. Proper PPE must be carefully chosen to mitigate the hazards presented by the agents and procedures used. To assist in the selection of appropriate PPE, workers should consult agent summary statements (64), agent manuals (9), material safety data sheets (when handling hazardous or potentially hazardous chemicals), facility standard operating procedures, and persons knowledgeable about the associated hazards, such as facility safety personnel. As a minimum, with consideration of the risks involved, PPE may include street attire protected by a full-length, long-sleeved, fully fastened laboratory coat, gown, or smock; closed-toe shoes; eye protection; ear protection; molded "surgical type" masks (filtering facepiece); appropriate gloves ("examination" or "surgical" type depending upon the need for sterile procedures); and HEPA-filtered respirators. Although HEPA filters used in respirators are not certified by the National Institute of Occupational Safety and Health (NIOSH) for use in a biological environment (65), these filters have been successfully used to protect personnel for many years.



Personal protective measures also include elements of an occupational health program. One element is medical surveillance, during which a physician determines if an individual is medically qualified to work with potential occupational hazards. Another element is the collection and storage of baseline serum samples, when appropriate, from individuals handling certain hazardous agents, or those participating in a special immunizations program. The occupational health program should evaluate the physical and mental suitability of personnel for duty assignment in areas where certain hazardous agents are handled. Another element of the program is to vaccinate at-risk personnel, those who may be occupationally exposed to certain agents. If a safe vaccine is available for the agent(s) being used and is known to protect against the agent(s), it should be provided for those individuals working directly with the agent(s) and for other at-risk personnel (such as facility maintenance, animal care, and safety personnel). The program should also include a mechanism for the immediate reporting of potential exposures to agents, such as mishaps, spills, or other accidents. It is extremely important to also report any illness that may be associated with working with these agents, no matter how remote the possibility of exposure may seem or even if an incident cannot be associated with the illness. Each exposure, or potential exposure, should be evaluated by appropriate medical staff and, if required, treated. Medical treatment of individuals potentially exposed to biosafety level 2, 3, or 4 agents can be safely accomplished by standard or transmission-based (airborne, droplet, or contact) precautions where appropriate (31, 32). The availability of a quarantine facility with a capability for isolation and medical care of personnel with a potential or known laboratory-associated exposure is required for Army facilities handling biosafety level 4 (maximum containment) agents (84).

## DECONTAMINATION

Decontamination procedures in a laboratory or research environment will vary depending upon the capabilities of the facility and the agent(s) being used (7, 20, 47, 68, 69). Decontamination is disinfection or sterilization of articles containing etiologic agents (microorganisms or toxins) to make them safe for use or disposal. Disinfection is the selective elimination of certain undesirable microorganisms to prevent their transmission. Sterilization is the complete destruction of microbial life (5, 20, 69).

Decontamination procedures can be discussed from two approaches: surface decontamination and area (space) decontamination. For surface decontamination, the effectiveness of a decontaminant depends upon decontaminant concentration, the concentration of the agent, type of agent, time of contact, and the environmental conditions (38, 43, 70, 87). There are a number of general groups of decontaminants such as alcohols, halogens, quaternary ammonium compounds, phenolics, and glutaraldehyde. Many of these decontaminants (1) are only active against certain groups of microorganisms while inactive against others. These decontaminants are used primarily for the interior of safety cabinets, room surface washdowns,

wiping off the exterior of certain items being removed from laboratories, and in chemical disinfectant showers used to disinfect a positive-pressure protective suit. Some of the halogen-containing decontaminants are corrosive. There is an association between autoclave corrosion and the use of halogenated disinfectants, particularly chlorine disinfectants. Chlorine may combine with organic materials during autoclaving. The resulting compound, or compounds, is corrosive in the autoclaves, drains, vents, and the central vacuum and receiver system that supports the autoclaves. The activity of chlorine-containing disinfectants, such as sodium hypochlorite solution, can be neutralized with sodium thiosulfate.

The second approach to decontamination is area or space decontamination, that is, decontamination of equipment or materials within enclosed spaces. This can be achieved by using a variety of gases (55). Ethylene oxide (epoxyethane, ETO) is a flammable and explosive gas, classified as both a mutagen and a carcinogen. The microbicidal activity of ETO is due to alkylation of sulfhydryl, amino, carboxy, phenolic, and hydroxyl groups in the spore or vegetative cell. The reaction of ETO with nucleic acids is the primary mechanism of its bactericidal and sporicidal activity. ETO is used because of its ability to inactivate most bacteria, molds, yeasts, and viruses, but its use is limited because of the many dangers mentioned. Propylene oxide (epoxy propane) hydrolyzes in the presence of moisture to form propylene glycol, which is nontoxic. Propylene glycol vapor (58) is odorless, tasteless, and nonirritating to the respiratory mucosa. The microbicidal mode of action of propylene oxide is the alkylation of DNA guanines resulting in single-strand breaks. Beta-propiolactone (BPL) is approximately 4000 times more active than ETO and 25 times more effective than formaldehyde. The microbiological activity of BPL is due to alkylation of DNA. However, there is limited use of this decontaminant because BPL lacks the ability to penetrate material and is carcinogenic in mice. Formaldehyde is more widely recognized as a fumigant for buildings and rooms (73, 79). Formaldehyde gas is capable of killing microorganisms and detoxifying *Clostridium botulinum* toxin. The microbicidal activity of formaldehyde is due to denaturation of proteins. Ammonium bicarbonate can be used to neutralize formaldehyde gas. Although formaldehyde vapor is explosive at concentrations between 7.0%–73.0% by volume in air, these concentrations should not be reached if standard decontamination procedures (using 0.3–0.6 g/ft<sup>3</sup> of paraformaldehyde in the presence of 60%–90% relative humidity) are used. The most commonly recommended decontaminant for an area is formaldehyde. It is used to decontaminate biological safety cabinets (25), laboratory rooms, laboratory areas, and equipment in airlocks. Although widely used and recommended as a surface and area sterilant, formaldehyde is a safety hazard because it is a potential occupational carcinogen. In addition, it is a powerful reducing agent, has limited penetrating ability, and is potentially explosive. Environmental release of formaldehyde is also highly regulated. For these reasons, technologies that may provide alternative sterilants are emerging. One alternative is the powerful oxidant chlorine dioxide, which is an effective sterilant even at concentrations as low as 20 mg/l. A relative humidity of 50% or higher is optimal for sterilization. Another

alternative, ozone, is not a new sterilant; it was used to sterilize the water supply of Lille, France, in 1899. It has potential as a sterilant for medical devices because it is highly oxidizing. The alternative gas plasma sterilization process uses radio frequency energy and hydrogen peroxide vapor to create a low-temperature hydrogen peroxide gas plasma to achieve relatively rapid sterilization. Radio waves break apart the hydrogen peroxide vapor into reactive species (free radicals), which form a gas plasma that interacts with and kills microorganisms. The advantage of this process is that the process temperature does not exceed 40°C. A relatively new alternative sterilization system is based on the vapor phase of hydrogen peroxide (6). The system provides a rapid, low-temperature technique that, because of its low toxicity, eliminates much of the potential public health hazard associated with decontaminants such as formaldehyde and ethylene oxide. In the cold sterilization process, 30% liquid hydrogen peroxide (300,000 ppm) is vaporized to yield 700–1200 ppm. The hydroxyl radical, a strong oxidant, is believed to have microbicidal activity through attack on membrane lipids, DNA, and other essential cell components. The hydrogen peroxide vapor is unstable and degrades to the non-toxic residues of water vapor and oxygen. Any sealable enclosure, such as small rooms, airlocks, biological safety cabinets, glove boxes, and isolation equipment, (up to 1,200 ft<sup>3</sup>) can be sterilized. The process is effective at temperatures ranging from 4°C to 80°C. The vapor phase hydrogen peroxide sterilization system appears to be safe and is effective against a variety of microorganisms. The range of etiologic agents inactivated by various decontamination techniques is extensively documented (1, 7, 47, 55, 58, 68, 69, 73, 87).

## PROTECTION DURING A RESPONSE TO AN INCIDENT

During a response to an incident involving biological agents, or during work in the field, it is anticipated that only personal protective measures and equipment and decontamination procedures may be available to personnel. Protection against biological agents can usually be provided by PPE used to protect against hazardous chemicals. As in a laboratory or research environment, it is important to consider the hazard when selecting PPE in a field environment. The availability and procedures for the use of protective equipment by military personnel against biological agents in a combat environment is well documented (13, 24). Personnel trained in microbiology and biological safety are essential resources in providing support to law enforcement agencies in response to an act(s) of bioterrorism or incident involving biological weapons. These professionals will be able to apply the principles of biosafety in a practical approach to the terrorism incident. An immediate evaluation of the situation is foremost and is accomplished by employing risk management procedures following the guidelines for conducting a risk assessment (4, 26, 33, 71, 81). Risk management is the systematic application of policies, practices, and resources to the assessment and control of risk affecting human health, human safety, and the environment. Risk assessment is an expression

of potential loss in terms of hazard severity, accident probability, and exposure to the hazard. One must consider the severity of the hazard by assessing the expected consequence, which is defined as the degree of injury or occupational illness that could occur from the hazard. The probability of an accident or illness occurring after a given exposure to the hazard must also be determined. Finally, exposure to the hazard considers the number of persons exposed and the duration or frequency of the exposure. A risk assessment will provide guidance for choosing the appropriate PPE for responding personnel and subsequent management of those individuals potentially exposed to a threat agent. A risk assessment is never completed. It is a constant review of implemented procedures, policies, and plans. Risk assessment is a method for reducing all hazards to the minimal acceptable level.

Levels of protection for a hazardous chemical incident (Table 1) are categorized from levels A (maximum protection) through D (limited protection). Level A provides the highest level of respiratory, skin, and eye protection. Level B provides the highest level of respiratory protection but less skin protection. Level C is used when the concentration and identification of the air contaminant is known so that an appropriate NIOSH approved air-purifying respirator can be used. Level D protection can be used where the atmosphere is free of all known hazards and the tasks do not pose a splash, immersion, or potential respiratory hazard. Level D is comparable to PPE worn in a hospital laboratory, that is, a hospital gown or laboratory coat, goggles, surgical mask, and latex examination or surgical gloves. Depending on the hazard, protection afforded by Level D or Level C may be adequate for emergency response personnel (67) or in a field environment. Where the hazard lingers and is concentrated and may be volatile, protection against biological agents can be provided by employing standard precautions or barrier nursing

**TABLE 1** Levels of protection—hazardous chemical incident

<b>Level of protection</b>	<b>Description of protective equipment</b>
A	Self-contained breathing apparatus (SCBA) or supplied air with a NIOSH approved escape pack and a fully encapsulated suit and undergarment with appropriate protective gloves, shoes, and head cover.
B	SCBA or supplied air with an escape pack, a chemical-resistant suit, and appropriate protective gloves, shoes, and head cover.
C	Chemical-resistant suit is used when it is known that the contaminant will not adversely affect exposed skin or be absorbed through the skin. Appropriate protective gloves, shoes, and head cover are used. Use of a face shield, goggles, or safety glasses with side shields is optional.
D	Escape mask, if required, and a work uniform or coveralls and protective gloves and shoes. Increased protection can be afforded with head protection, a face shield, goggles, or safety glasses with side shields. [Hospital gown or laboratory coat, goggles, surgical mask, and latex examination or surgical gloves.]

techniques depending on the biological agent (23, 37, 65). Individuals will be protected against the potential of person-to-person transmission of the agent(s) and any environmental biological hazards. The rationale is that biological agents, in most situations, would most likely be dispersed from the area before the arrival of personnel (35, 66). However, additional precautions such as respiratory and cutaneous protection are recommended in certain instances where smallpox, *Y. pestis*, the hemorrhagic fever viruses, or T-2 mycotoxin are suspected, and where there may be a hazard of person-to-person transmission, transmission by direct contact with blood or body fluids, or dermal activity (27, 28, 49, 65, 86).

Protection against biological warfare agents during a response to an incident, or in a field environment, involving biological agents can also be accomplished by implementing decontamination procedures (20, 36, 67) that employ mechanical (physical) and chemical methods. The technical decontamination process for gross biological contamination is used to decontaminate vehicles and PPE. The medical or patient decontamination process is for cleaning injured or exposed individuals (67). The sequential nine-step technical decontamination process, used by the public service community, was developed in 1995 (51). Depending upon the contaminant, four cleaning solutions (known as A, B, C, and D) are used with water to effect decontamination. For technical biological decontamination, a 10% solution of sodium hypochlorite (Solution B) is used (67). A 10% solution of calcium hypochlorite is used as Solution B (52) because an effective concentration can be achieved in addition to a longer shelf life than that of sodium hypochlorite (M. S. Hildebrand, personal communication). Rosen (67) & Noll et al (52) describe details of this technical decontamination process. Decontamination of equipment or fabric clothing is accomplished with a 30-min contact with 5% sodium hypochlorite solution, followed by cleaning with soap and water. Because this procedure is corrosive to metal and fabrics, a thorough rinse with water is recommended after decontamination, followed by a process to preserve the treated item (20). Patient or medical decontamination is required when the biological agent (contaminant) places the exposed individual, or patient, at further risk or presents a potential secondary risk to other personnel. The emerging consensus (16, 40, 60) is that decontamination of persons exposed to a potential biological aerosol is probably unnecessary and that, at most, clothing removal and a soap and water shower are perfectly adequate to prevent secondary exposures. Based on the available evidence (36, 40), reaerosolization of biological agents from clothing or skin does not appear to be a major issue. Healthcare providers and first responders are not at risk from such a hazard in most circumstances.

The efficacy of decontamination of inanimate surfaces with liquid household bleach is documented in recent experiments conducted at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) (Hawley & Nash, unpublished observations). Undiluted liquid household bleach inactivated 99.8% of the spore population (a 5 log<sub>10</sub> reduction in viability) of *B. anthracis* after 1 min of contact time. Similarly, after 1 min of contact, an *E. coli* population was completely inactivated (a 6 log<sub>10</sub> reduction in viability). *E. coli* was used as a gram-negative model in these experiments to simulate *Y. pestis* and other gram-negative bacteria

that are more fastidious in their growth requirements and are more dangerous to handle. Further experiments were conducted to determine the efficacy of 0.26% sodium hypochlorite (a 1:20 dilution of liquid household bleach, 2,625 parts per million free, available chlorine). Results showed a 100% inactivation of the spore population (a 5 log<sub>10</sub> reduction in viability) of *B. anthracis* after 15 min of contact time to 0.26% sodium hypochlorite. Using 0.5% sodium hypochlorite (a 1:9.5 dilution of liquid household bleach, about 5,500 parts per million free, available chlorine), we observed a greater than 90% inactivation of the spore population (up to a 3 log<sub>10</sub> reduction in viability) of *B. anthracis* after 5 minutes of contact time. Experiments are in progress to refine the contact time data for inactivating *B. anthracis* spores and to determine the influence of extraneous organic material on inactivation kinetics.

After an aerosol incident with a biological agent, the evolving belief is that there will be very minimal contamination of the clothing or skin of victims. In addition, decontaminating victims can be accomplished (if even necessary) by removing their clothing and having those potentially exposed shower at home with soap and water (16, 37, 40, 60). Reaerosolization of a biological agent in a hospital setting is unlikely (16, 23, 50, 65). The essential resources in providing support to first responders, law enforcement agencies, and the medical community to incidents involving biological weapons include microbiologists, biosafety professionals with a strong foundation in microbiology, and a designated clinical microbiology laboratory (72). The continuing education of medical responders is also an important component of this response network (14, 16, 56).

## RESPONSE ISSUES

In a field environment or during a response to a biological weapons incident, however, only personal protective measures, equipment, and decontamination procedures may be available to personnel. The Centers for Disease Control and Prevention (CDC) developed a strategic plan to address the deliberate dissemination of biological or chemical agents to reduce the vulnerability of the United States to biological and chemical terrorism. The plan includes preparedness planning, detection and surveillance, laboratory analysis, emergency response, and communication systems. For the diagnosis and characterization of biological and chemical agents, the CDC and its partners will create a multilevel laboratory response network for bioterrorism. The purpose is to link clinical laboratories to public health agencies in all states, districts, selected cities and countries, and to state-of-the-art facilities that can analyze biological agents. The CDC will transfer diagnostic technology to state health laboratories and others who will perform initial testing. They will also create an in-house rapid-response and advanced technology laboratory to provide around-the-clock diagnostic confirmatory and reference support for terrorism response teams (39). "Local" clinical microbiology laboratories will play an essential role in the initial recognition of a biological weapons

incident. The laboratory capability will be challenged to apply procedures for the isolation, rapid detection, and subsequent identification (66) of a potential threat agent(s) in a suspect sample(s). Sample analysis data from the clinical microbiology laboratory will provide guidance to first responders and healthcare providers for environmental and expedient patient management, respectively. The medical community must be responsive to any biological weapons incident. Their response will include a prompt identification of the biological agent(s) by the clinical laboratory, or laboratory response network, and notification of local, state, and federal health and law enforcement agencies. They must also be able to provide support to healthcare providers who may be overwhelmed with caring for large numbers of infected or intoxicated casualties (42). In point of fact, medical examiners and coroners may be the first to recognize unusual deaths due to a biological weapons incident before the medical community becomes involved (53).

Both the microbiologist and biological safety professional should be able to apply most of the principles of biosafety and provide a practical assessment of the biological weapons incident situation to responsible officials. Their knowledge is crucially important in helping to address microbiology and safety issues, minimizing fear and concerns of those responding to the incident, and helping to manage individuals potentially exposed to a threat agent. The biological safety professional will be able to apply the principles of biosafety in a practical approach to an incident involving a biological weapons agent.

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