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The controversy over genetically modified (GM) crops continues to be reported in the news and scientific journals. Proponents of GM crops cite benefits that include increased yield, nutrient enrichment, and resistance to pests and diseases. Opponents cite risks that include uncertainties regarding food safety, contamination of native crop species with genetic elements from GM crops, and danger to biodiversity. To learn more about considerations in assessing the risks of GM crops read "A Proposed Risk Assessment Method for Genetically Modified Plants" by Katia Regina Evaristo de Jesus et al., on pages 127-137. The pictures are (clockwise from top left): native maize (courtesy of CIMMYT), "golden" rice paddy, abundant mandioca harvest, tomatoes (is it GM or natural...), and, in the center, an abstract of maize genetics.
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- January 20 for Spring issue
- April 20 for Summer issue
- July 20 for Fall issue
- October 15 for Winter issue

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ABSA, the leader in the profession of biological safety.

Mission Statement

The American Biological Safety Association is dedicated to expanding biological safety awareness to prevent adverse occupational and environmental impact from biohazards.

Goals

• Expand professional and public awareness of biological safety through effective communication.
• Participate in the development of biological safety and biosecurity standards, guidelines, and regulations.
• Develop ABSA as the recognized resource for professional and scientific expertise in biological safety and biosecurity.
• Advance biological safety as a scientific discipline through education, research, and professional development.

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Edward John Stygar, III
I recently returned from a service visit to a busy Metro Toronto laboratory involved in the manufacturing of fine chemicals. During my visit, I was disappointed to observe at least 30 fume hoods within the facility that were not equipped with proper airflow monitors. As well, all of the laboratory’s newer fume hoods, which came from the manufacturer equipped with alarms, had their safety devices unplugged and disabled.

As a fume hood specialist with a manufacturer of fume hoods, I am constantly faced with this issue and the laboratory technicians’ and, ultimately, owners’ apparent disregard for safety.

It seems hard to believe that anyone would be willing to work without this important safety device, especially considering there are several safety guidelines published within the industry requiring properly functioning airflow monitors on all fume hoods.

I would like to take this opportunity to remind laboratory managers and safety officers that they should always specify fume hood alarms on new purchases and encourage laboratories to retrofit alarms whenever required. It is also prudent for laboratories to require employee training on the proper use of fume hoods when equipment upgrades are made. Most fume hood manufacturers are equipped to provide this service when required.

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**Editorial Note**

Letters to the Editors (approximately 400 words) discuss information published in *Applied Biosafety* in the past nine months or discuss topic areas of general interest in the biosafety profession. Letters can be submitted electronically to Karen D. Savage, Production Editor, at ksavage@covad.net or by mail to ABSA National Office, *Applied Biosafety*, 1200 Allanson Road, Mundelein, IL 60060. Letters published in part or whole are subject to editing for clarity and special formatting.

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**CDC Meeting Summary**

Submitted by Elizabeth Gilman Duane, Wyeth, Cambridge, MA

On March 7-8, 2006, several members of ABSA were invited participants at a Select Agent Biosecurity meeting convened by the Centers for Disease Control and Prevention (CDC) and facilitated by the Association of Public Health Laboratories. The goal of the meeting was to seek input from participants on how to provide more information on Select Agent Security Plan compliance for registered facilities and those applying for registration. The meeting, which was held in Atlanta, began with presentations covering Risk Assessment, Chain of Custody, Select Agent Security in Public Health and Academia, and Incident Response. The remainder of the meeting was dedicated to four focus groups that were tasked with evaluating certain aspects of the Security Section of the Select Agent regulations as well as draft guidance documents. Given that the Select Agent regulation is primarily a performance standard many registrants have had questions related to compliance. The 4 focus groups included: Risk Assessment, Risk Mitigation, Administration and Recordkeeping, and Guidance Documents. At the conclusion of the meeting each focus group reported on their findings and discussions. A publication of the meeting findings is slated to be completed 60 days from the conclusion of the meeting. The American Biological Safety Association appreciated the opportunity to participate.
In the previous issue of Applied Biosafety (Volume 11, Number 2, 2006), I explained the role of ABSA and its partners in the development of an internationally recognized biorisk management standard for laboratories. This program will take place through a series of European Committee for Standardization (CEN) workshops, resulting in a formal document known as a CEN Workshop Agreement (CWA). I would like to take this opportunity to update you on our progress.

A decision from the European Commission regarding CEN Workshop funding is imminent. All indications are very positive, but the devil is in the details. We won’t know the specifics until we see the contract. In anticipation of a successful funding initiative, we have taken our first steps toward the process.

We recently completed a workshop hosted by Dr. Stefan Wagener and the Public Health Agency of Canada in Winnipeg, Manitoba. It was attended by three representatives from Canada and one each from China, Scotland (Det Norske Veritas [DNV]), Britain (EBSA), Australia, Geneva (WHO), Sweden (EBSA), Singapore (A-PBA), and the U.S. (ABSA). We drafted a skeleton outline and a few descriptive examples of the possible text of a working document that might be used as a tool for the first CEN Workshop. When funding is approved and we have the go-ahead, we will enlist your help in providing a critical review of these and potentially other documents. As we will have only one year from the date of funding to hold three or four workshops and finalize the CWA, we hope to hit the ground running. Remember to send me a short e-mail (biosafety@mcn.org) stating or reconfirming your interest in being part of this review team.

I’ve been asked what the benefits of this voluntary standard will be. Originally, I thought that most American institutions would have little use for it. Then I remembered that one of the most common questions I’m asked by many colleagues is, “I know we practice biosafety, but it’s not well documented; do I need a formal biosafety program and, if so, what should it actually look like?” If we have a CWA, we could simply provide them this standard and say, “Here are the management requirements for such a program.”

In the longer view of benefits, some countries are in the process of developing biosafety as a national requirement, but have no solid guidance for creating the basic program. Other countries and institutions would like to develop biorisk management programs but are, for whatever reasons, reluctant to use the current American, Canadian, or “Western” documents as guidance. It is our hope that this CWA will, with its basis in the WHO Laboratory Biosafety Manual and its international derivation, serve as an universally-accepted guidance document.

Another clear benefit is that the CWA will be based on a management system, rather than a set of technical performance-based criteria. It will allow the creation of a biosafety program in which technical elements are approached as the program developer is able, rather than as he must in order to meet specific technical performance criteria. Countries or institutions, where money is so scarce as to preclude adoption of our often-expensive Western controls, are more likely to develop a program that allows them to meet basic biosafety requirements as they are able. The important part is that they must understand and define the risks; and with the help of a Guidance Document, they will have suggestions and examples of approaches. The final specific technical approach is, however, left up to the program developer.

It is important to remember that the focus right now is on writing the standard itself; the certification process is NOT part of this effort and will be addressed by another group at another time. No consideration is being given here about whether institutions self-certify or hire a certifier; if certification is through national or private certification agencies; if certifiers are trained in the process; who is to do that training? ABSA may ultimately become a certifying agency, or an organization that trains certifiers, or both. So may EBSA, DNV, or private commissioning agents. When the certification process is defined, all those wishing to qualify for a role will need to jump through the same hoops. Neither DNV, nor ABSA, nor EBSA, have an inside track on becoming a player in the ultimate process. At this time, we are simply facilitating the development of the standard that you, the biosafety professionals of the world, will write.

On another note, one of the Task Forces I appointed earlier this year has already submitted its final report to Council, two months ahead of schedule. The Philanthropic Activities Task Force, under the excellent leadership of Craig Welence, has presented Council with a prioritized list of philanthropic activities ABSA could provide to foster the awareness, evolution, and practice of biosafety in the U.S. and abroad. This Task Force also
developed a step-by-step set of proposal evaluation criteria that will ensure each philanthropic proposal, regardless of size or complexity, is afforded the same careful consideration. Council has unanimously agreed to appoint a new committee to oversee the implementation of the excellent recommendations of Craig’s team. As we become an increasingly recognized force in the field of biosafety, I believe we will see ever increasing numbers of proposals for ways we can contribute, at our own expense, to the evolution of our profession. I believe we have an obligation to undertake these efforts as we’re able, and this Task Force has given us the means to get started. I extend my sincere thanks to Craig and his excellent team for this fine work. The Council and I look forward to receiving the reports and recommendations of our other four Task Forces.

I have two things to ask of you, as members of ABSA. If you have not yet completed the member survey, please do so at your earliest convenience. Just go to the Members Only section of the web site and click on “Take the Members Survey.” While we have over 1,000 members, we’ve received less than 200 responses to the survey. We’re getting very interesting information, some of which you’ll hear about in Boston and may be useful to you when you seek professional development or advancement from your employers. Please add your information to the growing set of data.

Finally, pay close attention to our journal, Applied Biosafety. Its quality and utility have been growing steadily, thanks to our two superb co-editors and their editorial panels. It is now available online, as is the article submission process. We are currently assembling an electronic library and undertaking some translation efforts. Look for more great things to happen with Applied Biosafety.

In this past year of changes and challenges, I have had the privilege of working with many outstanding individuals, both on and off Council. Without the assistance and guidance of Betsy Gilman Duane, our Past-President, and Bob Hawley, our President-Elect, my job would have been much more difficult, and I thank them both for their dedication and support. I also tip my hat to our Councilors—a hard-working, committed group of professionals who help keep Council sharply focused on the health and welfare of ABSA. Many thanks to them all, and a special note of thanks to our departing Secretary, Rosamond Rutledge-Burns, and our two departing Council members, Patty Olinger and Chris Thompson, for their hard work. And last, but hardly least, a big “thank you” to Ed John Stygar, our Executive Director, and his tireless staff; without their support, dedication, and just plain hard work, our management transition would not have happened so seamlessly, nor would we be in such good hands today.

I feel very honored not only to have been your President this past year, but also to have had such wonderful people to work with. The next year will have its own unique set of challenges. Rest assured your Council and staff will keep ABSA strong, healthy, and successful.

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**EPA Pesticide Program Update: Data Requirements for Biochemical and Microbial Pesticides**

Date of publication: March 8, 2006

Citation: Federal Register, Volume 71, Number 45, Page 12071-12117

www.epa.gov/fedrgstr/EPA-PEST/2006/March/Day-08/p2185.htm

Purpose: EPA is proposing to update and revise its data requirements for the registration of microbial and biochemical pesticide products to reflect current scientific knowledge. These proposed revisions are intended to provide EPA with data and other information necessary to support the registration of a biochemical and microbial pesticide product, and will improve the Agency’s ability to make regulatory decisions about the human health and environmental effects of these pesticide products. EPA is also proposing to update the definitions of a biochemical pesticide and a microbial pesticide to more accurately describe these categories of pesticides, and to make a conforming change to the definition of microbial pesticide. EPA is announcing its policy to provide assistance to applicants when needed in determining what data are appropriate to support registration of a biochemical or microbial pesticide and encouraging applicants to request pre-submission meetings to discuss these data issues. EPA is announcing its intent to provide assistance to applicants in some narrow circumstances in preparation of an applicant’s data waiver.

Chemical(s): Various
Comments: Comments, identified by docket identification (ID) number EPA-HQ-OPP-2004-0415, must be received on or before June 6, 2006.

Contact: Candace Brassard or Nathanael Martin, telephone: 703-305-6598 or 703-305-6475, e-mail: brassard.candace@epa.gov or martin.nathanael@epa.gov
Abstract

One of the most important toxin threats in warfare or bioterrorism is Staphylococcal enterotoxin B (SEB), an incapacitating toxin. SEB had been considered and produced as an offensive biologic warfare agent. Staphylococcal enterotoxin B is a toxin associated with incidences of massive food poisoning. The bacteria that produce this toxin (SEB) are universally associated with man and other warm blooded mammals and their spheres of environmental influence include sewage and plumes. Staphylococcus aureus can readily be isolated from nose, armpits or anal swabs and about 50% of clinical isolates produce this toxin. Staphylococcal enterotoxin B is one of the superantigens capable of massive nonspecific activation of the immune system including a massive release of cytokines, such as interferon-gamma, interleukin-6 and tumor necrosis factor-alpha. Staphylococcal enterotoxin B is a potential agent of bioterrorism because of the ease of its production and dispersion, a delayed onset of symptoms, an ability to cause high morbidity and the difficulty in discerning between intentional intoxication and natural intoxication when a viable organism is the etiologic agent. This article presents a brief discussion on the recognition, management and surveillance of SEB, as well as the pathogenesis, clinical manifestation, diagnosis, and treatment of patients exposed to this toxin.

Introduction

In the 1960s, the USA had an offensive biological warfare program and SEB was one of the agents studied as a biological agent that could be used to incapacitate soldiers in the battlefield. This was an attractive agent because low quantities were required to affect the desired incapacitation when compared with chemicals synthesized in the laboratory (Ulrich et al., 1997).

With the establishment of the Department of Homeland Security after the September 11, 2001 attacks, administrative officers in the Homeland Security have recognized that bioterrorists can use any weapon to carry out their threat. It is important to be mindful of the ordinary symptoms of unusual human and animal diseases and report them to local security authorities as quickly as possible. Delays in recognition and subsequent reporting of bioterrorism can mean the difference in life and death for literally thousands of humans and animals.

Many biological agents and toxins can cause illness in humans, but not all are capable of effecting public health and medical infrastructure on a large scale. The public health infrastructure must be equipped to quickly resolve crises that would arise from a biological or chemical attack. Toxins, chemical compounds synthesized in nature by living organisms, can be classified by molecular weight, source, preferred targets in the body and mechanisms of actions. Many factors place practical limits on their use as mass casualty weapons. These factors include—production, delivery, and environmental stability and host factors (Madsen, 2001). Terrorist use of SEB might be manifested as deliberate contamination of food and water. Therefore the aim of our review is to discuss further the various avenues that SEB could be used as a biological weapon.

The Toxin

The Staphylococcal enterotoxin B (SEB) comprises a large group of proteins produced by several species of bacteria including Staphylococcus, Streptococcus and Mycoplasma (Bergesll, 1970 & 1979). Staphylococcal enterotoxin B is responsible for a number of extensive pathophysiological changes in humans and mammals and triggers an excessive cellular immune response leading to toxic shock (Kaempfer, 2004). Staphylococcal enterotoxin B, together with ricin and epsilon toxins, is classified as category B Priority Pathogens by National Institute for Allergy and Infectious Diseases (CDC, 2000).

S. aureus are found in all foods that have been handled by humans or that have been contaminated by animal matter. They grow well in most prepared food, including meats, vegetables, fruits, pastries, and milk prod-
ucts (Vela, 1997). In the laboratory, S. aureus grows well on nutrient agar containing about 10% sodium chloride. *Staphylococcus aureus*, with *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and enteropathogenic *Escherichia coli* are responsible for more than 90% of food poisoning cases each year in USA (Vela, 1997). Exotoxins (proteins) and related pyrogenic toxins from these bacteria easily diffuse out of the cell (Novick et al., 2001). A distant related protein to SEB, toxic shock syndrome toxin-1 (TSST-1), also produced by *Staphylococcus aureus*, was isolated in the early 1980s and is responsible for the induction of tampon-related toxic shock (Novick et al., 2001). Staphylococcal enterotoxin B is a part of a set of exotoxins produced by *S. aureus* which comprise about 15 antigenically distinct proteins and include the following: SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, SEH, SEG, SEI, SEJ, SEK and the last one discovered recently was identified as SEU (Lefertre et al., 2003). Many of these toxins are closely related and are collectively called superantigens because they interact with the immune system to activate a very high percentage of T-cells (Michelke et al., 1992). Various studies have shown that not all these toxins play a role in food poisoning (Lefertre et al., 2003). According to the information provided, all but two SE's cause gastrointestinal (GI) symptoms (Su & Wong, 1995; McLauchlin et al., 2000; and Omoe et al., 2002).

Staphylococcal enterotoxin B could pose a great risk to consumer health and can be classified as a low molecular protein (24 - 29KD) (Su & Wong, 1997). Because of low molecular weight, SEB could induce gastroenteric symptoms which include diarrhea and vomiting in humans, and as superantigens, may also cause toxic symptoms (Marreck & Kappler, 1990) by initiating the activation and proliferation of T-cells with certain Vβ (variable domain of T-cell receptor β-chain) regions on their T-cell receptor (Michelke et al., 1992). If mature animals are exposed to SEB, mature T-cells bearing target Vβ's respond to the challenge by rapid proliferation and production of cytokines (Kotsin et al., 1993). SEB Symptoms can be induced by as little as 30ng (Kotsin et al., 1993). Experiments have shown that for aerosol exposure, the effective dose, or ED50 (dose capable of incapacitating 50% of the exposed human population), is 0.0004mcg/kg, and the lethal dose, or LD50, is .02mcg/kg (Rusnak et al., 2004). The extremely small amount of material that is required for toxic effect indicates that a complex is necessary for the toxin to exert its effect. SEB can represent a practical bioterrorist weapon because purified toxin can be isolated from *S. aureus* culture supernatants.

**Mechanism of Action**

*Staphylococcus* enterotoxin B must first enter the body and gain access to immune cells to do any harm. SEB binds to major histocompatibility complexes (MHC) class II molecules and stimulates T-cells by binding to T-cell antigen receptors with strong avidity, independent of antigen recognition (Figure 1). Up to one in five T-cells may be activated, whereas only one in 10,000 is stimulated during a usual antigen presentation. When these T-cells are stimulated, an immediate activation and prolif-

---

**Figure 1**

Superantigens and the non-specific stimulation of T-cells: Superantigens (SEB) bind directly to class II major histocompatibility complexes (MHC II) of antigen-presenting cells outside the normal antigen binding groove. Up to one in five T-cells may be activated. Cytokines are released in large amounts, causing the symptoms of toxic effects of SEB. Figure by C. Alexander Designs. All rights reserved.
erations of the T-cells with \( \beta \)'s variable domain of T-cell receptor will ensue (Kappler et al., 1989). When exposed to SEB, mature T-cells bearing target \( \beta \)'s (variable domain of T-cells receptor \( \beta \)-chain) respond to the challenge by rapid proliferations and productions of cytokines which are thought to mediate most of the toxic effects of SEB (Marrack et al. 1990, Stiles et al. 2001). So engagement of class II molecules by the toxin on macrophages or mast cells stimulates these cells and causes release of soluble mediators beneficial to the host in small quantities. SEB binds directly to class II major histocompatibility complexes of antigen-presenting cells outside the conventional antigen-binding groove. This complex recognizes only the \( \beta \) element (variable domain of T-cells receptor \( \beta \)-chain) of the T cell receptor. Thus, any T cell with the appropriate \( \beta \) element can be stimulated, whereas normally, antigen specificity is also required in binding (Kotzin et al., 1993).

### Symptoms

Symptoms of SEB intoxication include a sudden onset of fever, about 40°C to 41°C, chills, headache, myalgia, and a non-productive cough. Some patients may develop shortness of breath and chest pain. Fever may last 2-5 days and cough may continue for up to one month. Patients may also present with nausea, vomiting, and diarrhea when the toxin is swallowed (Ulrich et al., 1997). The effects on those exposed while under stress, such as soldiers in combat situations, may appear to be much more severe. This exposure can result in vasodilation and pathological drop in blood pressure, respiratory distress, shock and death within 40-60 hours of exposure. Some forms of SEB-intoxication result from absorption of the toxin into circulation from mucosa surfaces (gut) (Ulrich et al., 1997). In a recent finding, individuals working in a laboratory were diagnosed with conjunctivitis with periorcular or facial swelling as a result of ocular or cutaneous exposure. This was the first report of eye irritation involving SEB. This emphasizes the importance of face masks and eye protection for those individuals working with SEB (Rusnak et al., 2004). Although SEB is not generally considered lethal, high levels of exposure can lead to septic shock and death.

### Pathogenesis and Clinical Manifestation

Staphylococcal enterotoxin B symptoms occurring in humans is associated with the site of entry. When the toxin is ingested, this will result in the inflammation of the gut leading to diarrhea and vomiting. If the toxin is absorbed through the dermis, there is an inflammation of the skin resulting in dermatitis and delayed type hypersensitivity (DTH) (Rusnak et al., 2004). However, when the eye is infected, there is an inflammation of the eye resulting in iritis. In inhalation of SE, there is a sudden onset of fever, headache, chills, myalgia and a non-productive cough. In more severe cases, the patient may develop dyspnea and retrosternal chest pain leading to the inflammation of the lung and respiratory distress (Ulrich et al., 1997). When the toxin is absorbed into the circulation, there is inflammation of the vasculature resulting in toxic shock (McLauchlin et al., 2000). Two hours after intoxication, patients with SEB typically begin to experience blurred vision, headache, abdominal distress, diarrhea, and vomiting and generalized body weakness (Rusnak et al., 2004). Medical treatment is not prescribed for SEB intoxication unless there is excessive loss of electrolytes from vomiting and diarrhea. In this case, electrolyte replacement and treatment of symptoms are the only measures indicated.

### Epidemiology

The unfortunate fact remains that humans are often the most sensitive detector of a biological attack (Ulrich et al., 1997). Without the knowledge of the attack, an increased number of patients presenting with signs and symptoms caused by the disseminated disease agent is the first indicator that SEB exposure has occurred. SEB is not contagious and cannot be transmitted from person to person. In contrast, when this toxin is expressed in E. coli, the toxin produced is very potent (Kotzin et al., 1993). No instances of waterborne toxin contamination have ever been reported, although the potency of SEB has led to speculation that it might be used to contaminate a municipal water supply. If food were deliberately contaminated and used as a carrier, the outbreak would need to be distinguished from naturally-occurring foodborne Staphylococcus food poisoning. Staphylococcal food poisoning is quite common in the USA and all other countries of the world. Outbreaks are numerous during all seasons of the year, with a noticeable increase during the summer months. The rapidity of onset and severity of SEB intoxication depends on the rate and amount of toxin absorbed. When the toxin is ingested through food, symptoms may begin as soon as two hours after ingestion (Kotzin et al., 1993). Symptoms may last as long as 12 hours then disappear completely. Normally, recovery is uneventful and no residual effects remain even after severe intoxication. Rusnak et al., reviewed occupational exposure to SEB and concluded that the knowledge of full clinical spectrum of SEB intoxication is important to healthcare workers evaluating persons with potential exposure to SEB and including in the context of bioterrorism (Rusnak et al., 2004). Any outbreak of SEB should bring to mind the possibility of bioterrorism, but certain features would be particularly suggestive such as multiple simultaneous outbreaks with no common source (Rusnak et al., 2004).
Detection of SEB

Methods for fast detection and identification of SEB are highly desired to provide early information to healthcare providers and safety officers in the event of a bioterrorist attack. In recent years, rapid progress has occurred in the area of biosensor development. Should SEB be used as a bioterrorist agent, one should expect exposure by inhalation or contamination of food and water. On the battlefield, where the SEB will be distributed as aerosol, the device will have specific detection needs. For example, this device should be automatic, unattended, and remote or carried as an analytical test system to be used under battlefield conditions. In this context, the device could be used to analyze such samples as air, water, personnel, and equipment. Menking & Goode (1993) described the detection of SEB using the light addressable potentiometer sensor (LAPS). Using this method, a lower limit of SEB (2ng/mL) was detected. SEB has also been detected with an impedance-based immunosensor (DeSilva et al., 1995). Tempelman et al., (1995) reported the use of a fiber optic biosensor for the detection of SEB on a variety of clinical, environment and military samples. King et al., 1999 used the Man-portable Analyte Identification System (MANTIS) which is the first fully automated, self-contained, portable fiber optic biosensor for the detection of SEB. This device detected SEB spiked into liquid samples with no false positives and could perform simultaneous immunoassays rapidly in the field with little or no intervention by the user. Homola et al., 2001 have reported the use of Surface Plasmon Resonance (SPR) which is a wavelength modulation-based sensor to detect SEB in milk. This sensor was able to detect SEB at low concentration of 5ng/mL without amplification. This report also indicated that SPR could be tailored for the detection of various food pathogens. Over the past few years, multiple PCR assays and multiplex PCR assays which detect specific gene sequences for SE’s and TSST-1 by DNA amplification have been developed (McLauchlin et al., 2000, Schmitz et al., 1998, Sharma et al., 2000). This real-time PCR appears to be a much more efficient method because it allows for the analysis of large number of samples at the same time, thereby saving more time than the conventional PCR and does not detect any false positives. Finally, 24 hours after exposure to SEB, the toxin could be identified from nasal swabs from individuals exposed by aerosol. This may be important in the battlefield since this can be used as an early diagnosis.

Biosafety and Decontamination

With increased funding for biodefense research and many institutions working with SEB, it is possible there will be an increase in laboratory exposure and intoxication with SEB. It is necessary to document the symptoms of SEB intoxication in order to educate healthcare workers and safety officers to enable them to properly identify those individuals at risk and thereby prevent exposures to SEB. Biosafety is the measure intended to prevent accidental release of SEB from a research facility that could endanger the public and environment. Biosafety is achieved through use of primary and secondary containment devices such as Biological Safety Cabinets (BSC), good laboratory practice/technique and glove boxes. These barriers protect the researcher from the toxin (SEB) while the filters prevent the toxin (SEB) from entering the environment. In a report addressing the issue of biological safety cabinets’ (BSC) efficacy, a contractor noted that individuals working with SEB on laboratory benches without BSC experienced toxic reactions (Wendum, 1996). Staphylococcal enterotoxin B is a bio-toxin that can be acquired by inhalation, ingestion, or injection. The toxin is a highly-soluble protein that is easily removed with soap and water and inactivated by autoclaving. After exposure to SEB, clothing, skin, and eye should be washed thoroughly with soap and water for at least 15 minutes. Contaminated surfaces (for example laboratory tables and BSCs) should be cleaned with disinfectant solution and contaminated objects secured and autoclaved.

Biosecurity

The Centers for Disease Control and Prevention (CDC) and Animal Plant Health Inspection Service (APHIS) have provided a model list that could be followed by facilities as a basis for biosecurity standards (42 CFR, Part 73.11). In this list, the CDC has grouped agents of bioterrorism into three categories (A, B, and C) depending on their impact on public health and environment. Staphylococcal enterotoxin B is a category B agent, which is moderately easy to disseminate, and, if exposed within the civilian population, will result in moderate morbidity and low mortality rates. Most of these agents in category B that could be used for bio-terrorist acts may be obtained from sources such as patients and infected animals. Therefore, a terrorist could have access to these sources and be able to isolate the agents in order to use them as a weapon of mass casualty. The probability of a terrorist having the technical expertise and skills required to isolate and culture these organisms is very low. The greater risk would be the terrorist stealing the agent from a research laboratory or purchasing the agent from a national culture collection or a commercial supplier under false pretense. The essential provision of a biosecurity measure is to make it difficult for the terrorist to acquire these agents, ensuring that researchers are performing legitimate research, and research facilities are off limits to individuals who have not gone through FBI security screening. The biosafety regulation determines who has
access to the agents, what agents (SEB) the entity possesses, and the locations of the facilities using these agents (CFR 42 Part 73). Therefore, any facility that has acquired these agents (SEB) is required, by regulation, to conduct threat, vulnerability, and risk assessment. Since it is difficult to obtain a quantitative accounting of all the agents of bioterrorism, it is important to develop a security plan tailored specifically to the agent in use (in this case SEB) and the unique characteristic of the agent (ABSA, 2002). Any security plan should reference the CDC revised and expanded guidelines entitled “Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents” (Richmond et al., 2002). Other forms of security required by the regulation include inventory controls in order to track internal possession and transfers, and inactivation and disposal of culture after use. Physical security is designed to impede unauthorized entry into the laboratory in order to steal the select agent (SEB) stored within the facility. This security should be at the level of hazard or threat associated with the agent (in this case SEB). In order to provide security and protection for facilities researching with SEB, all the guidance provided above needs to be followed precisely.

Treatment

The American experience on September 11, 2001 has accelerated the demand for the development of therapies and vaccines against various agents of bioterrorism. Treatment for SEB is administering the victim supportive medical care to minimize the effect of the intoxication. The supportive medical care would depend on several factors, such as the route by which the victims were poisoned (that is, either by inhalation, ingestion, or skin or eye exposure). Medical care needed at this time may include helping the victim breathe, giving the victim intravenous fluid and flushing their stomach. For oral exposure, washing out mouth with water and if swallowed, vomiting should be induced. Historically, SEB vaccine research which focused on formalin-inactivated toxin (Silverman et al., 1969, Tseng et al., 1993) has been carried out by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). This vaccine, produced by prolonged incubation in formalin, was evaluated for its ability to induce protective antibodies in monkeys by intracutaneous (Silverman et al., 1969), intramuscular (Tseng et al., 1993), intratracheal (Tseng et al., 1995), and intragastric (Tseng et al., 1995) routes. This vaccine was shown to be immunogenic when delivered parenterally with no clinical side effects and stimulates protective antibody responses (Silverman et al., 1969). However, SEB toxoid is a poor immunogen when given nasally but, in combination with protosomes and biodegradable microspheres, it stimulates antibody responses. USAMRIID abandoned this vaccine despite its good attributes in favor of a recombinant vaccine which uses a site-directed mutant (Boles et al., 2003, Boles et al., 2003). These vaccines were designed with the knowledge of molecular interactions between SEB and MHC II/Vβ TCR. Mantis (2005) has indicated that a vaccination regimen for humans should be carefully optimized since immunized animals (5µg/dose) showed lower rate of survival. In an experiment in which mice were immunized orally or intranasally with SEB triple mutant vaccine in combination with cholera toxin as adjuvant, anti-SEB IgA antibodies were stimulated in serum and salivary secretions (Stiles et al., 2001). Research in recombinant vaccine will ultimately lead to the development of safe, effective vaccines that can be distributed through oral, intranasal or transcutaneous routes which will be capable of inducing both systemic and local immunity.

Conclusion

The use of SEB as a weapon of mass casualty is considered likely for several reasons, mainly high morbidity with ease of production and dispersion, the delayed onset of disease symptoms associated with high morbidity and low mortality and difficulty in diagnosis. Staphylococcal enterotoxin B is a superantigen capable of massive nonspecific activation of the immune system. Because of the remarkable toxicity and stability, they would most likely be disseminated as an aerosol, in food, or water supplies. Several vaccine trials in animal models appear to be promising but, in order to perform these trials in human subjects, it will be necessary to understand which receptors are used to attach and penetrate the epithelial barrier, the effects of SEB on mucosal cells and role of mucosal immunity. In the context of bioterrorism, this review will be relevant to military personnel considering that SEB is an incapacitating biowarfare toxin.

References


Center for Disease Control and Prevention. (2000). Biological and Chemical Terrorism; Strategic Plans for Preparedness and Response. MMWR, 49, 1-14.


Staphylococcal Enterotoxin B as a Biological Weapon


Fact Sheets on Terrorist Attacks

The U.S. National Academies of Science has prepared fact sheets to provide reporters with reliable information on biological, chemical, nuclear, and radiological attacks. This effort was a collaboration with the U.S. Department of Homeland Security, and the Radio and Television News Directors Foundation. ABSA members may find the information useful in educational efforts on emergency planning.

The fact sheets can be found at www.nae.edu/factsheets.

Biological Attack (pdf file, 277 KB)—Where do biological agents originate? What’s the difference between “infectious” and "contagious”? How long after exposure will symptoms appear?

Chemical Attack (pdf file, 72 KB)—What are the different origins of toxic chemicals that could be used? How do chemical toxicities vary? What are the practical steps to take if there’s a chemical release?

Radiological Attack (pdf file, 68 KB)—What are radiological dispersal devices, a.k.a. “dirty bombs”? How are they different from nuclear bombs? What are their physical and psychological health effects?

Nuclear Attack (pdf file, 192 KB) NEW!—What is radioactive fallout, and how is it dangerous? What are the short-term and long-term effects of radiation exposure? What is the likely size of a nuclear explosion from an attack by terrorists?
A Proposed Risk Assessment Method for Genetically Modified Plants

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Abstract

An essential step in the development of products based on genetically modified plants (GMPs) is an assessment of safety, including an evaluation of the potential impact of the crop and practices related to its cultivation on the environment and human or animal health. The purpose of this safety assessment is to compare information about the GMP with that from a non-GM crop. However, at present this risk analysis may be faulty because there is no widely accepted and specific risk assessment method to evaluate GMPs that uses quantifiable parameters and allows for a comparative analysis among different technologies. This paper introduces a risk analysis method that focuses on the identification and evaluation of risks associated with the field release and cultivation of GMPs. Two tools bolster this proposed risk assessment method: (1) worksheets to compile Evidence of Risks, and (2) a Matrix of Assessment. The first tool identifies potential hazards related to the use of a specific GMP. This preformatted worksheet assigns values to the level of risk and its significance in terms of the activity to be developed. The second tool provides a structure to observe the potential hazards that illustrates what approach supports the use of GMPs in a manner as safe as any other traditional technology. To better understand this proposed risk assessment method, it is presented in a digital format (www.cnjma.embrapa.br/forms/gmp_ram.php³) (GMP-RAM v.1.1. software) where the two tools are linked so that the user can fill in the worksheets and automatically observe the results in the matrix. Compared to current processes, this proposed method represents a less subjective and more transparent process for risk assessment.

Introduction

An essential step in the development of a genetically modified plant (GMP) is the assessment of its safety. This procedure evaluates all possible influences of the plant and practices related to its cultivation on the environment and on plant, human, and animal health. This is accomplished by comparing appropriate comparators, for example, wild genotypes of the plant used to produce the GMP. This evaluation is performed by risk analysis.

Risk analysis follows a structured approach with three distinct but closely related steps: risk assessment, risk management, and risk communication. In addition, factors related to risk prevention, reduction, and remediation should also be considered. Following Codex Alimentarius, noted in the Food and Agriculture Organization’s biotechnology glossary (FAO, 2001), risk assessment is defined as “a scientifically based process consisting of the following steps: (i) hazard identification; (ii) hazard characterization; (iii) exposure assessment and (iv) risk characterization.” Risk management is “the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practice, and, if needed, selecting appropriate prevention and control options.” Risk communication is defined as “the interactive exchange of information and opinions throughout the risk analysis process concerning risks, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.”

The literature describes several ways to complete a risk analysis (Conner et al., 2003; EFSA, 2004; FAO, 2003; FAO, 2004; Krayer von Krauss et al., 2004). The evaluation is scientifically based, where parameters that comprise a risk (such as hazard and exposure) are submitted to qualitative analysis (Funtowicz et al., 1999; NRC, 2002; OECD, 2005). However, there is no widely accepted and specific risk assessment method for the evaluation of genetically modified plants that draws on quantifiable parameters and allows for a comparative analysis among different technologies.

This paper introduces a risk analysis method, focused
on the risk assessment step that identifies and evaluates the risks associated with the release and cultivation of GMPs. The proposed method is based on other methods of risk analysis, such as Ambitec-Agro (Rodrigues et al., 2003a), Environmental Assessment System (Rodrigues et al., 2003b), Matrix of Leopold (Leopold, 1971), and Environmental Impact Assessment methods utilized during ISO 14000 implementation. Many validated issues or parameters of analysis described in previous reports (EFSA, 2004; NAS, 2002) were also considered.

The uniqueness of this risk assessment is the assignment of values for specific parameters; these values make it possible to describe and compare risk measurements with quantifiable tools. The hazards to be analyzed are organized according to their potential sources of exposure, such as genetic insert, expressed protein, features of the GMP, gene flow, introduction of the technology, and unexpected occurrences (accidents). All concerns related to GMPs, or at least the currently most debated ones, can be arranged in these groups.

All activities related to commercial release, field trial tests, greenhouse experiments, or even lab assays with GMPs are assessed with this proposed method. Therefore, it can be used throughout GMP development, from the researcher during new trait search to the regulators during assessment for market clearance. Obviously, the exchange of information and experience among all involved allows for an accurate analysis of GMP safety.

Although some risk assessment methods already propose how to weight the evidence of risks (EC, 2002; EFSA, 2004), they do not allow the user to find out which are the most relevant issues that should be managed during the risk analysis. In contrast, the risk assessment method presented in this paper introduces tools that give the user the opportunity to identify and rank potential hazards; consequently, mitigations are made according to the context of the risks.

**Methods**

The proposed new risk assessment methodology is performed in two steps: (1) Complete a preformatted worksheet to compile the evidence of risks, and (2) Plot the outcome on the Matrix of Assessment.

**Evidence of Risk Worksheet**

First, a worksheet is constructed to characterize all potential GMP-related hazards and to assign a level of risk and its significance in the context of the activity to be developed. Table 1 shows the worksheet, and the following topics describe the worksheet's different fields.

**Fields for Sources of Exposure, Potential Hazards, and Criteria of Assessment**

On the worksheet the potential hazards are grouped according to their source of exposure, along with at least one criterion for assessment of each one. These items are predetermined on the worksheet to allow for an accurate evaluation of related risks. For example, altering the dynamic of population of weeds is a "potential hazard" resulting from the gene flow that is the "source of exposure." The characterization of this hazard is performed by analyzing the "criterion of assessment" that is the feasible outcrossing between the GMP and the related weeds.

The data presented in the worksheet are based on features of current GMP traits (James, 2004; OECD, 2005). In addition, public concerns about genetically modified organisms and the impact of environmental assessment were also considered when deciding which issues to analyze in the worksheet (MAFF, 2000; NAS, 2002; SCIENTISTS, 2002). However, new features and genetic characteristics have been developed, resulting in new potential hazards, sources of exposure, and criteria for assessment. Thus, new aspects must be added to the worksheets as needed.

In Table 1 each hazard is coded with a letter (from “a” to “o”) to identify it in the Matrix of Assessment. The user may also add parameters to the tool (e.g., potential hazards can be inserted in rows coded “p” to “z”) according to the specificity of the technology and on a case-by-case basis. Likewise, it is possible not to complete the parameters that are not related to the object under analysis.

**Data/Information for the Evaluation Field**

This central column is the field where the user describes all information related to the criterion of assessment. Experimental results and literature searches must be the source of the scientific data described. For example, in the previous example where the criterion of assessment was the outcrossing between GMP and related weeds, the user could cite the sexual compatibility, distance and rate of outcrossing, barriers for pollen containment, and the usual isolation distance in seed production as data for evaluation. These data are crucial to support the risk characterization since the assignment of values must correspond with the information described by the user in the corresponding field.

**Indexes of Moderation Fields**

Based on scientific data, the risk characterization is accomplished by attributing values separately for two specific indexes: Index of Risk and Index of Significance. These comprise the "Factors of Moderation," such as damage, exposure, precedent, extent, and reversibility.

(1) Index of Risk: This index is calculated taking into account the factors below:

Index of Risk = Damage x Exposure x Precedent

• **Damage:** Level or intensity of the impact (damage)
### Table 1

**Worksheets for the Compilation of the Evidence of Risk**

“Potential hazards” are listed according to their “potential source of exposure.” For each identified hazard, at least one criterion to assess it is listed. Risk characterization is performed by attributing values to the “factors of moderation” and, consequently, estimating values to the “indexes of moderation.” The fields in the central column are to be filled in with current scientific data that supports the values assigned to each factor of moderation.

<table>
<thead>
<tr>
<th>Potential Hazards</th>
<th>Criteria for Assessment</th>
<th>Data/Information for Evaluation</th>
<th>Factors of Moderation</th>
<th>Factors of Moderation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Damage</td>
<td>Exposure</td>
</tr>
<tr>
<td><strong>Potential Source of Exposure – Genetic Insert</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Dissemination of diseases, development of antibiotic resistance</td>
<td>Donor features</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Recipient features</td>
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<tr>
<td></td>
<td>Presence of unwanted or regulatory or marker nucleotide sequences that present some risk</td>
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<tr>
<td>(b) Appearance of other negative characteristics</td>
<td>Stability of the insert</td>
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<tr>
<td></td>
<td>Phenotypic and compositional assessment, pest and disease reactions</td>
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<tr>
<td><strong>Potential Source of Exposure – Expressed Protein</strong></td>
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<tr>
<td>(c) Occurrence of negative effects on plant, human or animal health</td>
<td>Protein specificity</td>
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<td></td>
<td>Homology with known allergenic or toxic proteins</td>
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<td></td>
<td>Protein stability</td>
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<tr>
<td>(d) Occurrence of negative effects on nontarget organisms</td>
<td>Protein toxicity or allergenicity for non-target organisms</td>
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<tr>
<td><strong>Potential Source of Exposure – Features of GM Plant</strong></td>
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<tr>
<td>(e) Generation of plants with weedy aspects</td>
<td>Recipient features</td>
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<td></td>
<td>Reproductive, competitive, or adaptive ability</td>
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<tr>
<td>(f) Outbreak of additional attributes</td>
<td>Gene specificity</td>
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</tbody>
</table>

*Table 1 is continued on the next page.*
Table 1 (Continued)
Worksheets for the Compilation of the Evidence of Risk

<table>
<thead>
<tr>
<th>Potential Hazards</th>
<th>Criteria for Assessment</th>
<th>Data/Information for Evaluation</th>
<th>Factors of Moderation</th>
<th>Factors of Moderation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Damage</td>
<td>Exposure</td>
</tr>
<tr>
<td><strong>Potential Source of Exposure – Gene Flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Unexpected dissemination due to outcrossing with conventional genotype</td>
<td>Rate and distance of outcrossing</td>
<td></td>
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<tr>
<td>(h) Alteration in the population distribution of weed or native species</td>
<td>Outcrossing with weed or wild species</td>
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<tr>
<td>(i) Alteration in other nontarget organisms</td>
<td>Gene flow to nontarget organisms (horizontal flow)</td>
<td></td>
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<tr>
<td><strong>Potential Source of Exposure – Introduction of the Technology</strong></td>
<td></td>
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<tr>
<td>(j) Threat to the agricultural management practices</td>
<td>Comparative analysis with conventional methods</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(k) Decreases in the efficacy of the technology</td>
<td>Probability to generate mutations in the target organisms</td>
<td></td>
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<tr>
<td>(l) Increasing the demand for natural resources</td>
<td>Comparative analysis regarding soil and water usage and quality</td>
<td></td>
<td></td>
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<tr>
<td><strong>Potential Source of Exposure – Unexpected Occurrences (accident)</strong></td>
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</tr>
<tr>
<td>(m) GMO dissemination due to extreme climatic events</td>
<td>Geographic relationship in the region to sensitive areas</td>
<td>Localization of the site (e.g., position of field trial inside the property)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n) GMO dissemination due to operational failure</td>
<td>Training of the team</td>
<td>Final destination of the GMO regulations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(o) Dissemination due to theft of material</td>
<td>Physical safety of the property</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
that the GMP could have on the system, if the proposed adverse effect actually occurs. The intensity (low, medium, or high) is quantified according to the values in Table 2.

- **Exposure**: This is related to the level that some component (e.g., soil, animal, native plant, etc.) is exposed to the damage. The higher the exposure the higher the possibility of an adverse effect occurring. The values attributed to different levels of exposure are shown in Table 2.

- **Precedent**: Precedent considers the previous occurrence of the adverse effect, as a consequence of the event in question, as shown in Table 2.

Based on the definition of Index of Risk, the score may range from 1 (1x1x1) to 32 (4x4x2); therefore, the Index of Risk is able to classify a risk as very low, low, medium, and high, as seen in Table 2.

(2) Index of Significance: For additional characterization, the risks must be evaluated according to the context of the activity to be developed. For example, the Index of Significance takes into account the location where the GMP will be cultivated, the identification and evaluation of potential adverse effects, and the evaluation of the current environmental situation. This index is calculated by:

- **Extent**: This factor of moderation reflects the extent of dispersal or the distribution of the damage, classified as shown in Table 2.

- **Reversibility**: This is the ability of the system to return to the previous condition (considering the activity with GMPs). The values to quantify this factor are shown in Table 2.

Extent and Reversibility are factors of moderation that directly affect decisions on how to manage actions for risk mitigation or risk prevention. The Index of Significance also ranges from 1 (1x1) to 32 (4x8), being classified from “very low” to “high,” as shown in Table 3.

The potential hazards identified with the letters (a), (b), (c), (e), (m), and (n) present more than one Criterion of Assessment. In these cases, it is suggested that the higher value among the criteria be selected to measure the indexes. This ensures a high confidence for the proposed method.

**Matrix of Assessment**

After hazard identification, hazard characterization, exposure assessment, and the significance analysis of related risks comes the final step in this risk assessment

### Table 2

Values to be attributed to the Factors of Moderation that Comprise the Index of Risk (damage, exposure, and precedent) and the Index of Significance (extent and reversibility).

<table>
<thead>
<tr>
<th>Factors of Moderation</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>4</td>
</tr>
<tr>
<td>Exposure</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>4</td>
</tr>
<tr>
<td>Precedent</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>Extent</td>
<td>Local (contained where GMP is cultivated)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Regional (property or distance of pollination)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Abroad (area affected indirectly)</td>
<td>4</td>
</tr>
<tr>
<td>Reversibility</td>
<td>Naturally reversible (without management)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Reversible with simple management (e.g., changing technology)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Reversible with complex management (high costs and use of nonconventional methods)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irreversible</td>
<td>8</td>
</tr>
</tbody>
</table>
process—reviewing the potential hazards and establishing at which level risk management, by preventive or corrective actions, must be taken in order to allow safe use of the GMPs. This is performed by the Matrix of Assessment step.

The Matrix (Figure 1) is constructed with two axes, where the “x” axis stands for the classes of the Index of Risk and the “y” axis stands for the classes of the Index of Significance. The results from the Index of Risk and the Index of Significance are plotted in the Matrix according to their position (points are plotted using letters that represent each potential hazard). The level of mitigation recommended is classified as:

(a) No restrictions—when the hazard does not have a significant chance of being a risk
(b) Monitoring required—when the hazard must be observed to avoid adverse effects
(c) Management required—when additional measures must be taken to prevent impacts
(d) Restrictions required—when the activity can be done under restrictive rules or measures and, additionally, frequent observations are required to avoid potential impacts
(e) Not recommended—when hazards show a high level of risk and significance. In this case biosafety measures could be ineffective to prevent or mitigate such risks.

Compiled Analysis

The following step involves compiling and analyzing the results from the matrix and worksheets. Each potential hazard plotted in the matrix requires some measures according to the level of mitigation. These biosafety measures must consider all data described in the worksheet, such as the specificity of the GMP, the activity under analysis, and the environmental situation.

Essentially, this compilation is the core structure to perform risk management.

Digital Format—Introduction of the Software GMP–RAM (v. 1.1)

The software GMP–RAM v. 1.1 is an electronic format of the worksheets that was created in Borland Delphi 2005 Professional and can be accessed via two different links: (1) www.cnpmaembrapa.br/forms/gmp_ram.php3 (risk assessment file to download) (preferable) and (2) ftp://ftp.cbi.cnptia.embrapa.br/ or www.cbi.cnptia.embrapa.br/gmp_ram/ (alternative). To run the GMP-RAM, just download the file to your PC and execute it with a left mouse double click. By using this electronic format, it is possible to attribute the values for the factors of moderation and the results of the indexes (Risk and Significance). These will be calculated and plotted in the Matrix of Assessment automatically.

Method Validation

This new risk assessment tool will be validated as soon as several users test it with different crops and traits or perform comparative analyses with other methods. It is expected that some fine-tune adjustments and/or improvements in the method will be needed.

Hypothetical Example

For better understanding Table 4 shows a hypothetical example of a partially completed worksheet and the corresponding Matrix of Assessment (Figure 2) plotted with the letters. The potential hazards characterized in this example were related to “unexpected occurrences” (coded with the letters “m”, “n,” and “o”).

<table>
<thead>
<tr>
<th>Risk</th>
<th>Index of Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>1-3</td>
</tr>
<tr>
<td>Low</td>
<td>4-7</td>
</tr>
<tr>
<td>Medium</td>
<td>8-15</td>
</tr>
<tr>
<td>High</td>
<td>16-32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance</th>
<th>Index of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>1-3</td>
</tr>
<tr>
<td>Low</td>
<td>4-7</td>
</tr>
<tr>
<td>Medium</td>
<td>8-15</td>
</tr>
<tr>
<td>High</td>
<td>16-32</td>
</tr>
</tbody>
</table>
Obviously, during a risk assessment of a GMP, the user must complete the whole worksheet. The assigned values for the factors of moderation are based on the information described in the worksheet (“data/information for evaluation” field) (Table 4). These do not describe the characteristics of the GMP, since this is not relevant at this point, but they do illustrate what we would consider if the GMP is a crop such as maize, cotton, or soybean and the activity to be developed is a field trial release.

Considering the distribution of the “letters” inside the matrix (Figure 2), the following risk management could be suggested:

- **Potential hazard coded as “m”** (GMO dissemination due to extreme climatic event)—Based on the information presented in the worksheet (Table 4), it seems highly feasible that the GM seeds could be undesirably disseminated as a consequence of flooding. The assessment of this potential hazard (“m”) indicates that it requires some “restrictions” to mitigate the risk. Considering that the more critical criterion of assessment was the “localization of the site,” it is mandatory that the field trial be installed at another site and not subjected to flooding or location near the river (or swamp).
- **Potential hazard coded as “n”** (GMO dissemination due to operational failure)—Assuming there is frequent biosafety training for the team and rules in place regarding discarding the material, this issue does not pose significant risk, so it does not require additional actions.
- **Potential hazard coded as “o”** (dissemination due to

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**Figure 1**
Matrix of Assessment is the final step of this Risk Assessment tool. The Matrix of Assessment gives an overview of potential hazards and establishes at which level risk management must be taken. The “x” axis represents the classes of the Index of Risk and the “y” axis represents the classes of the Index of Significance.
### Table 4
**Worksheet of Evidence of Risks Partially Filled-In**

This hypothetical example aims to show how to complete the worksheet during risk characterization. This example considered that the GMP is a crop (such as maize, cotton, or soybean) and the activity to be developed would be a field trial release. Only one “potential source of exposure” is to be filled in.

<table>
<thead>
<tr>
<th>Potential Hazards</th>
<th>Criteria for Assessment</th>
<th>Data/Information for Evaluation</th>
<th>Factors of Moderation</th>
<th>Factors of Moderation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Damage</td>
<td>Exposure</td>
</tr>
<tr>
<td>(m) GMO dissemination due to extreme climatic events</td>
<td>Geographic relationship in the region to sensitive areas</td>
<td>The property is located in a region target of storms and flooding</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Localization of the site (e.g., position of field trial inside the property)</td>
<td>The site for GMP cultivation is near to a river (swamp)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Period of occurrence of extreme climatic event</td>
<td>High pluviosity season does not match with planting season</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(n) GMO dissemination due to operational failure</td>
<td>Training of the team</td>
<td>The employees frequently attend biosafety courses running by expertises</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Final destination of the GMO regulations</td>
<td>There are specific rules that guide on how to discard the material resultant from GMP</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(o) Dissemination due to theft of material</td>
<td>Physical safety of the property</td>
<td>The area is easily accessed but there is police round service at night</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The level of mitigation of this issue was “management” because some failures in the security to access the property were observed. Evaluation of the data described in the worksheet suggests that safety measures must be implemented in the area. These would include a private security service and/or additional monitoring improvements.

Based on the recommendations and suggestions above, this hypothetical activity would be environmentally safe if the restrictions and managements above were duly implemented. The Matrix of Assessment presented in Figure 2 shows which potential hazards could affect the user’s choice of where to develop the GMP activities.
Conclusions

Risk analysis must be undertaken to predict the occurrence of negative impacts on the environment and human and/or animal health. These assessments allow us to define predictive measures to mitigate or avoid the adverse effects that could result from potential or identified hazards. Thus, it is possible to develop the GMP with a high probability of success and safety.

The risk assessment proposed here includes parameters that allow for an estimation of the level of risk based on the assignment of numeric values for several factors reported to correlate with risk. This results in lower subjectivity and higher transparency in the analysis processes. Recognition and characterization of risk significance (context of the risk) result in the definition of specific measures to be implemented, focusing on minimizing the chance of adverse effects.

NAS (2002) observed that a number of comparisons are appropriate for assessing the risks of transgenic crops. For example, the environmental effects of a transgenic crop could be compared to chemically intensive farming practices and to farming practices developed to be more ecologically sustainable.

In a risk assessment it is appropriate to draw on previous knowledge about the biology of the plant and to compare non-GM crops to the GM crop in order to highlight differences associated with the genetic modification and the subsequent management of the GM crop (EFSA, 2004). Technologies with the same objectives can also be compared using this proposed method, such as GM herbicide tolerant crop x mutant herbicide crop x traditional herbicide pulverization. The comparative

Figure 2
Hypothetical example of the Matrix of Assessment with plotted letters from Table 4.
analysis between conventional and genetically modified genotypes relies on the fact that all environmental regulations, such as isolation distance, water use management, etc., are duly followed. Therefore, the impact on preserved areas, bodies of water, etc., must be considered only if some specific GMP characteristics threaten such resources even when those regulations were respected.

Although this risk assessment is focused on GM plants, the range of the parameters analyzed in the risk characterization allow for the possibility of applying this method to the assessment of different technologies or activities, for example the introduction of an exotic plant species in a region or the cultivation of a conventionally improved crop for some abiotic stress-tolerance.

Considering the range of traits for GMP to be developed and the safety concerns that must be addressed on a case-by-case basis, this proposed method may not cover all aspects related to a given GMP, although it does present a broad approach to risk assessment. Since there is always the possibility of developing a new and better method that could be utilized in a wide range of situations, the user is encouraged to expand the possibilities of this tool by adding or deleting parameters (e.g., potential hazards) according to the specificity of the technology. On the other hand, regulators must assess if the chosen parameters are the best ones to define the potential impact of the technology under analysis.

This strategy is crucial to result in a less superficial method, since it is able to attribute which parameters are more correlated to the technology. In addition, characterizing risk by measuring with quantifiable tools demonstrates a quantitative method where subjectivity is drastically decreased.

Acknowledgements

The authors thank Dr. Donna Mitten and Dr. Magdalena Belaustegui for critical reading, as well as Dr. Mauro Zackiewicz (a consultant from Elabsis, the agency of computational support).

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Scientists Oppose BT Cotton/Anger Grows Over Failure to Stop GM Crop Test (1 May 2002). Available at: http://nginx.tripod.com/010502b.htm


Safety Library Reference

The National Academies Press provides, free of charge, Prudent Practices in the Laboratory: Handling and Disposal of Chemicals, 1995, in a searchable, printable format at:

www.nap.edu/openbook/0309052297/html/index.html

NIOSH Guide

NIOSH has an excellent new guide available for the prevention of occupational exposures to West Nile virus.

www.cdc.gov/niosh.docs/2006-115/#d
Laboratory Biosecurity: A Survey of the U.S. Bioscience Community

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Sandia National Laboratories, Albuquerque, New Mexico

Abstract

Laboratory biosecurity practices, or measures to prevent the theft or sabotage of biological research materials, must coexist with biosafety. Within the United States, laboratory biosecurity, for a list of select agents, has been regulated through several Codes of Federal Regulation. In 2004 and 2005, Sandia National Laboratories conducted a survey of the U.S. bioscience community in conjunction with Reed Research Group, to assess the extent biosecurity is implemented in laboratories and the relationship between biosecurity and biosafety and good laboratory practices in regulated select and non-select agent laboratories. This paper describes the results of this survey.

Introduction

In 1997, the first Select Agent Rule (42 CFR 73) went into effect, regulating the transfer of a small number of human pathogens. The United States significantly enhanced its regulatory approach for security of pathogens and toxins following the passage of the USA PATRIOT Act of 2001 and the Bioterrorism Preparedness Act of 2002. In February 2003, three interim Codes of Federal Regulations (CFRs) became effective which specified security measures for approximately 80 pathogens and toxins—now known as select agents—deemed to pose a threat to human (47 CFR 73), animal (9 CFR 121), or plant (7 CFR 331) populations. The regulations required any laboratory that possesses one of these agents or toxins to enforce and adhere to specific security measures, which include facility registration; designation of a responsible official; security risk assessments for individuals with access to the listed agents; laboratory biosecurity plans; agent transfer rules; safety and security training and inspections; notification after theft, loss, or release of a listed agent; record maintenance; and restrictions of certain experiments. The final rules, which replaced the interim regulations in March 2005, had only a few changes that impact the implementation of biosecurity at laboratories (CDC ABSA correspondence, 2005):
1. the term “access” is now defined as possession or the ability to gain possession;
2. notification of when a toxin or select agent is destroyed after transfer is no longer required;
3. laboratories are no longer required to keep records of when individuals exit areas with select agents; and
4. new requirement that biosecurity, biosafety, and incident response plans be exercised at least annually.
Most of the other changes were intended to harmonize the structure and format of the HHS and USDA regulations.
The final CFRs define the regulatory requirements for laboratory biosecurity in the U.S.; however, facilities that do not work with select agents (SA) are not required to implement these rules. This paper describes the results of a survey of the bioscience community in the United States. The Sandia National Laboratories (SNL) Biosecurity Team had three main goals in conducting this survey: 1) to understand the real and perceived positive and negative impacts of the interim CFRs on those facilities that work with select agents (SA respondents); 2) to understand how those facilities have implemented the required biosecurity; and 3) to learn what types of biosecurity measures, if any, are in place at facilities that work with pathogens and toxins that are not select agents (non-SA respondents).
This paper details the responses of both SA and non-SA respondents about the biosecurity measures in place at their facilities. This paper also briefly addresses the impact of the interim CFRs on bioscience facilities. Additional details on the respondents’ views of security of biological materials and the CFRs, positive and negative impacts of the CFRs, the inspection process, and the effect of the CFRs on domestic and international collaborations and recruitment of qualified individuals can be found in the survey data (available at www.biosecurity.sandia.gov/survey).

Survey Methodology

SNL worked with Reed Research Group (Reed Business Information, Newton, MA) to write and conduct a survey of the U.S. bioscience community. Reed used e-mail lists to solicit potential respondents who were then
directed to a web-based survey, and assisted SNL with refining the survey and collecting the first 222 responses. The SNL Biosecurity Team also collected additional data through a web-based version of the survey on its secure server. An additional 129 responses were received through the SNL web site. Responses to the survey on the SNL web site have been solicited through the posting of a link on the American Biological Safety Association (ABSA) web site and an announcement on the ABSA e-mail listserv. The SNL web-based survey was further publicized through presentations at the ABSA Annual Meeting in October 2004 and at a meeting sponsored by the Chemical and Biological Arms Control Institute and the International Institute for Strategic Studies in December 2004 (Rivera, 2004). The results presented in this paper reflect the aggregate responses from all of the above elicitations for a total of 351 respondents. This paper only discusses results received before March 2005 (prior to the issuing of the final CFRs). Unless otherwise stated, the given response percentages are based on the responses received to the specific questions.

Respondents were asked to answer the survey questions based on the following definitions of biosafety and biosecurity: Biosafety aims to reduce or eliminate exposure of individuals and the environment to potentially hazardous agents used in biological research, while biosecurity aims to protect dangerous pathogens and toxins, along with critical security-related information, from theft and sabotage by those who intend to pursue bioterrorism or biological weapons proliferation.

Results

A copy of the survey questions can be obtained from the SNL biosecurity web site (www.biosecurity.sandia.gov/survey). The site also has an Excel file with all of the survey responses (without identifying information) available for download. Survey responses represented a broad cross-section of bioscience institutions that work with select agents (180 SA respondents) and those that do not (171 non-SA respondents). According to Dr. Julie Gerberding’s testimony to the U.S. Congress on July 28, 2005, there are currently 333 entities registered with the Select Agent and Toxins Program (Gerberding, 2005). Of the SA respondents to this survey, 48.9% represented universities, 8.3% represented clinical or diagnostic facilities, 13.9% represented industry, 22.2% represented government facilities, and 6.7% identified as other. Of the non-SA respondents, 37.4% represented universities, 24.6% represented clinical or diagnostic facilities, 23.4% represented industry, 7.6% represented government facilities, and 7% identified as other. The respondents were also asked to identify the principal activities of their laboratories; they could select more than one response so the percentages do not necessarily equal 100%. The principal laboratory activities of the SA respondents were identified as basic research (57%), applied research (46%), and clinical or diagnostic work (33%), while the principal activities of non-SA respondents were clinical or diagnostic work (54%), basic research (39%), and applied research (31%).

All respondents were asked about their perceptions regarding the interim CFR. Thirty-one percent of non-SA and 19.4% of SA respondents answered that the interim CFR imposed prudent security measures. Ten percent of SA respondents and 7.6% of non-SA respondents believed the CFR were a good first step but didn’t go far enough; more security was needed. Over 40% of non-SA respondents and 50% of SA respondents believed that the interim CFR were on the right track but needed to be revised to provide clarity. SA respondents generally agreed that the greatest positive impact of the CFR is the increased awareness of the risks posed by some pathogens and toxins. However, the time and effort required by staff to comply with the regulations, and the inconvenience of increased security were frequently cited as negative impacts. Very few SA respondents believed there were no positive (8%) or negative (5%) impacts associated with the interim CFR.

The distribution between those respondents responsible for ensuring implementation of biosecurity (biosafety officers and responsible officials), those most affected by biosecurity measures (scientists and technicians), and directors/managers was balanced. Of all survey respondents, 23.6% were biosafety officers, 9.4% were responsible officials, 16% were principal investigators, 12.3% were laboratory support staff/technicians, 27.6% were directors/managers, and 11% were identified as other.

A facility risk assessment is the foundation of a good biosecurity program and this is acknowledged in the CFRs: “The security plan must be designed according to a site-specific risk assessment and must provide graded protection in accordance with the risk of the select agent or toxin, given its intended use.” Almost half of all SA respondents (48.4%) indicated that their facility’s biosafety officer conducted the risk assessment. Other facilities used multiple types of personnel to carry out the risk assessment (Figure 1) including security contractors (9.9%), local guard force (4.2%), and staff administrators (13.5%).

Management is responsible for implementing and overseeing a biosecurity program. Program management responsibilities include identifying the protection objectives, designing the security system, writing security and emergency response plans, conducting regular training and internal reviews, and allocating resources. Respondents were asked to identify what assets their security is designed to protect, people, property, or specifically pathogens and toxins. Of all SA respondents, 79% indicated that they have a security posture designed to protect...
people, 76% have a security posture designed to protect property, and 74% have a posture designed to specifically protect pathogens and toxins. In contrast, only a small number of the non-SA respondents have a security posture designed to protect people (14%), property (14.6%), or pathogens and toxins (10%). However, the question about the security posture had a low response rate (<40%) among non-SA respondents, suggesting that perhaps these respondents were not familiar with the rationale for their institutions' security.

Security planning and training were other areas explored in the survey. Even though the CFRs specifically require a written security plan, only 67% of SA respondents indicated they had a written plan. Of the non-SA respondents who implement biosecurity, 39.8% had a written security plan. Biosafety training is conducted at most facilities (95% of SA and 83% of non-SA), while biosecurity training is less common at both SA facilities (72.2%) and non-SA facilities (32.7%). We anticipated that few of the non-SA respondents would have biosecurity-specific training but it is surprising that not all of the SA facilities provide such training, especially since the CFRs require biosecurity training on an annual basis for all individuals with authorized access to select agents.

Standard elements of a biosecurity program include physical security (including access controls), personnel screening, material control and accountability measures (including inventories), transport security, and information security. As specified by the CFRs, these elements should be implemented in a graded manner based on the risk assessment. Access controls are typically considered to be an element of physical security. The CFRs require facilities to limit unescorted access to only those who have been authorized to work with specific select agents by either the Centers for Disease Control and Prevention (CDC) or the Animal and Plant Health Inspection Service (APHIS). This authorization is predicated on the successful completion of a security risk assessment process that is conducted by the U.S. Federal Bureau of Investigations (FBI). Almost all of the SA respondents indicated that access is controlled to their SA laboratories (Figure 2). Most of their facilities also control access to buildings and freezers. In contrast, few non-SA respondents claim to limit access to their buildings, laboratories, or freezers. The types of access controls that are commonly in place at SA facilities include: electronic access controls (81%), such as a badge swipe, proximity card, or personal identification number; guard identification (41%); and mechanical lock and key (52%). Biometric access controls, including retinal/eye scanners and fingerprint or hand-geometry readers, are only employed by 14% of SA facilities. In contrast, non-SA respondents indicated that their facilities relied more heavily on less sophisticated mechanisms to control access: 55% used mechanical keys and locks and 27.6% relied on guards to control access, while only 6.5% implemented electronic access controls and 4.6% used biometric access controls.

SA facilities handle access in emergency situations by different mechanisms. A few SA facilities reported they allow emergency workers to override the access controls (14.1%), some indicated emergency workers can override those controls while under the escort of an authorized person or after receiving permission from the institution (43.4%), and some stated they do not allow emergency workers to override the access controls. However, in most cases, access is controlled by electronic access controls or biometric access controls.
workers to override the access controls at all (42.5%). The survey questions did not provide any insight into the emergency response protocols of those facilities that do not allow emergency override of the access controls.

Many respondents also stated that they controlled access to freezers where select agents are stored. SA respondents generally controlled access to freezers by mechanical key and lock (70%), but many used electronic access controls (18.9%). Most non-SA respondents who controlled access to their freezers used mechanical key and lock (39.4%).

Escorting is a standard means to provide security when visitors and other unauthorized personnel need access to controlled areas. Eighty-nine percent of the SA respondents and 80% of non-SA respondents required visitors to be escorted. Nearly three-quarters of the respondents recognized that escorting contributes to both biosecurity and biosafety at their facilities (74% of SA respondents and 71% of non-SA respondents).

Identification badges are a common method for identifying individuals who work at a particular institution or who have authorized access to a specific area within an institution. Badges can also be used as a type of key, providing access into areas controlled by electronic access control systems. Nearly three-quarters (73%) of SA respondents’ facilities reported they require badges to be worn in the laboratories, except for biosafety considerations. Fifty-nine percent of non-SA respondents’ facilities indicated that they require badges to be worn in the laboratories. Badges were most often required to be worn at SA government institutions (93.3%), followed by clinical and diagnostic institutions (86.7%), industrial facilities (65.4%), and universities (61.4%).

Inventory systems, a measure that helps with material control and accountability, provide a mechanism for knowing what materials are stored and handled at a facility. Inventories are good laboratory practice and serve both biosafety and biosecurity goals. A majority of the SA respondents (65%) indicated they inventory select agents differently than non-select agents at their facilities. Approximately three-quarters of the SA respondents had inventories that track select agents in seed stocks and working stocks but only 26% track animals. Nearly three-quarters of the SA respondents tracked select agent materials by vials while 37% tracked Petri dishes and only 26% tracked animals. The majority of non-SA respondents also used their inventories to track seed stocks (73%) and working stocks (64%).

The CFRs require select agent facilities to include specific information in their inventory records for select agents that are held in long-term storage, including the name and characteristics (e.g., strain designation); quantity acquired (e.g., number of vials); date of acquisition; source; storage location; when moved from storage and by whom; when returned to storage and by whom; and, for toxins, quantity amount (e.g., milligrams). The interim CFRs also required information on disposal (date of disposal and quantity, volume, or mass destroyed or otherwise disposed of). The SNL Biosecurity Team wanted to understand how many of these inventory criteria are common laboratory practice, therefore the non-SA respondents were asked which type of information is tracked in their inventory systems. All of the inventory information required by the CFRs was reported to be

![Figure 2](image-url)

**Figure 2**
Areas of controlled access for SA and Non-SA respondents.
included in inventories of at least 40% of the non-SA respondents (Figure 3). This demonstrates, for inventory issues, the CFRs appear to be capturing standard laboratory practices.

Inventory systems can take many forms; they can be paper-based or electronic systems. Over 63% of all SA respondents maintained a paper-based inventory, such as log books, and approximately two-thirds utilized either electronic spreadsheets or electronic databases for inventory records. The percentages do not sum to 100% because many institutions used multiple inventory methods. The final CFRs require that “the individual or entity must implement a system to ensure that all records and databases created under this part are accurate, have controlled access, and that their authenticity may be verified.” The SNL Biosecurity Team’s survey responses were based on only the interim CFRs, which did not include a requirement for controlling access to records, such as inventories. Although access control was not a requirement of the CFRs, the survey did explore who had access to inventory records. Only 63% of the principal investigators (PIs) at SA facilities and 32% of the PIs at non-SA facilities had access to the inventory records. This is surprisingly low and may be due to a misinterpretation of the question by the respondents. The SNL Biosecurity Team anticipated a much higher percentage of PIs would have access to the inventory records since they would be responsible for maintaining the inventory of materials in their individual laboratories. Approximately 58% of biosafety officers and responsible officials at SA facilities also had access to the inventory records. In contrast, only 35% of biosafety officers at the non-SA facilities had such access. Only 2.2% of SA respondents did not limit access to these records, allowing anyone to access their inventories.

Conclusions

Approximately half of the respondents (181) identified themselves as Select Agent respondents, or respondents who work with or oversee research with select agents. Only 2.3% of all respondents answered the “security of pathogens and toxins is unnecessary,” implying that over 97% believe that security of pathogens and toxins is needed. This strong consensus about the need for biosecurity of pathogens and toxins provides a positive starting point for policy makers and those who must implement. Such community “buy-in” is essential for effective implementation of biosecurity. However, the support for the rationale of biosecurity does not necessarily translate to the “how” of biosecurity. The Office of Health and Safety of the Centers for Disease Control and Prevention has provided some initial guidance on implementing biosecurity; this report stressed that the “security plan should be an integral part of daily operations” (Richmond, 2002). For modern laboratories, biosafety and biosecurity jointly define the laboratory operating environment (Gaudioso, 2006) so it is critical to carefully plan the implementation of biosecurity to avoid conflicts with biosafety. The impacted community (e.g., scientists) will be less likely to comply with biosecurity measures that jeopardize or are perceived to jeopardize their safety.

The details highlight differences in the security postures between research institutes that work with select
agents and those that do not. For instance, the CFRs require access controls to select agent laboratories, and 85% of SA respondents reported they control access to their laboratories. Surprisingly, very few non-SA respondents reported use of access controls for the building (outside doors) or laboratory at their facilities. The survey results also indicated several areas of incomplete compliance with the CFRs by SA respondents. For instance, more than 30% of those respondents lack a written biosecurity plan. In contrast, the survey results suggest that inventory practices required by the CFRs are widely implemented in non-SA laboratories. Personnel security measures, such as escorting procedures and badges, are also used in both SA and non-SA facilities.

Biosecurity measures that are standard laboratory practice (as indicated by measures in place at non-SA facilities) may have greater community acceptance. However, successful implementation of biosecurity will require time since, as one respondent indicated, it requires adaptation, especially in settings “where a culture of free and open access exists. Some measures of biosecurity require a significant paradigm shift.” Many respondents recognized, “it’s important to involve safety, law enforcement, and researchers into the process of security plan development. Each group brings a unique perspective to the table.” Despite this recognition, implementation of biosecurity, including the facility risk assessment, is often the responsibility of the institution’s biosafety officer. Thus, it is imperative that more detailed technical guidance be provided to these individuals. The results of the survey provide a foundation for understanding the prospects of successful implementation of biosecurity measures, both domestically and internationally.

**Authors’ Note**

This work was created under U.S. Government contract by employees of Sandia National Laboratories as part of their official duties. The U.S. Government retains non-exclusive rights to use the work.

**References**

CDC correspondence to ABSA regarding the Final Rule. (March 18, 2005). Available at www.absa.org/word/050318CDC.doc


Chlorine Dioxide Gas Decontamination of Large Animal Hospital Intensive and Neonatal Care Units

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Abstract

In May of 2004, the large animal intensive care and neonatal intensive care units (ICU/NICU) of the University of Pennsylvania’s New Bolton Center were temporarily closed to all admissions following an outbreak of salmonellosis that had affected its patients. Environmental testing continued to recover a multi-drug resistant organism identified as Salmonella enterica serovar Newport following repeated liquid disinfection treatments of the facility. Based upon various environmental considerations, it was decided that the most feasible and effective process for disinfecting the facility would be achieved using chlorine dioxide (ClO2) gas. The facility was appropriately sealed, supplied with gas distribution mechanisms and demonstrated to have met the specification for humidity before introduction of ClO2. The total exposure for this 4800 cubic meters structure was approximately 400 ppm-hr. Efficacy of the decontamination was monitored by the placement of 20 Bacillus atrophaeus spore, 40 Geobacillus stearothermophilus spore, and 40 Salmonella Newport vegetative cell strips. Microbiological testing demonstrated greater than 5.5 and 6.1 log reduction for the G. stearothermophilus and B. atrophaeus spore strips, respectively. Log reductions of the S. Newport bacterial strips were also within acceptable levels. The success of this project demonstrates the utility of ClO2 gas as a biological decontaminant approach for mid-sized commercial and public facilities.

Introduction

The George D. Widener Large Animal Hospital, located within the New Bolton Center in Kennett Square, Pennsylvania, is one of the busiest, large animal hospitals in the United States, having approximately 6000 patient visits annually with a primarily (82%) equine caseload. As part of the University of Pennsylvania, this hospital also serves as an integral part within the University’s School of Veterinary Medicine and is important to the teaching, research, and service missions of the school. Subclinical and clinical infections with Salmonella are reasonably common in large animal patients (Dunowska et al., 2004; Morley et al., 2004; & Smith et al., 2004) and major outbreaks of nosocomial Salmonella infections have been documented at large animal veterinary teaching hospitals and other veterinary facilities over the past several decades (Anon., 2001; Castor et al., 1989; Dargatz & Traub-Dargatz, 2004; Hartmann et al., 1996; Parè et al., 1996; Schott et al., 2001; & Tillotson et al., 1997). Among equine patients, horses presenting with gastrointestinal disturbances, such as diarrhea and colic, may be at highest risk of shedding Salmonella (Ernst et al., 2004; House et al., 1999; Kim et al., 2001; Morley et al., 2004; & Palmer et al., 1985). As a tertiary care referral center, the Widener hospital admits a large number of critically ill and emergency cases. The largest proportion of emergency admissions, about half of approximately 1,200 emergency admissions per year from 1998-2003, have colic or diarrhea as their primary problem. As is the case with other large animal veterinary teaching hospitals, Widener Hospital is at greater risk of having animals that are actively shedding Salmonella within its environs than many other animal housing facilities.

A possible Salmonella outbreak was detected at the Widener Hospital in mid-March 2004. A weekly environmental surveillance program of various sites around the Widener Hospital had been employed for several years and would occasionally have a sample test positive for Salmonella. In response to the perception of increased cases, environmental and patient surveillance was increased in March, but gross contamination of the environment was still not indicated. A number of major steps were taken during the ensuing 58 days to determine the precise nature of the contamination and bring it under control. Surveillance of patients was increased to include submission of fecal samples from all animals (including
those with no clinical signs of infection) housed in high-risk areas. Efforts were made to clean and decontaminate high-risk areas while maintaining essential hospital services. Specific parts of the hospital were temporarily closed to patients. After all disposables were discarded, these areas were cleaned, disinfected and restocked before reopening. During these 58 days, the intensive care unit and neonatal intensive care unit (ICU/NICU) itself was subject to two such rounds of cleaning and disinfection.

In mid-April, the environmental monitoring and culture techniques employed were changed in two ways. First, electrostatic dust collection wipes (Swiffer®; Procter and Gamble, Cincinnati, OH) were used for sample collection (Burgess et al., 2004). The charge on the wipes attracts debris and bacteria and thus the wipes can be used to sample large surface areas. Secondly, a more sensitive technique for the isolation of Salmonella, utilized by the PADLS (Pennsylvania Animal Diagnostic Laboratory System) microbiology laboratories for isolating low numbers of stressed Salmonellae from environmental samples and foodstuffs, was adopted. This method was an adaptation of the International Standards Organization method for the detection of Salmonella spp. (ISO, 2002).

Toward the end of the 58-day period, the hospital was closed to elective in-patients; however, out-patients were seen, but only emergency cases were admitted to the hospital. Despite these extensive control efforts, culture-positive animals continued to be identified. Retrospective analysis of medical records revealed that between January 1, 2004, and closure of all parts of the hospital on May 10, 2004, 37 animals were positive for a group C2 Salmonella identified as Salmonella enterica serovar Newport. There was significant morbidity and mortality among the animals that were shedding Salmonella in their feces, although some of the culture-positive animals never showed clinical signs of salmonellosis.

During the last two weeks of April, 37 of 140 cultures taken throughout the hospital complex were found to be positive for S. Newport. The causative agent was identified as a multi-drug resistant form of S. Newport, a gram-negative, non-spore forming bacterium. In light of the positive fecal cultures obtained from patients and the environmental findings, it was deemed necessary in early May 2004 to discharge all remaining patients and close the entire Widener hospital until the adverse bacterial population could be brought under control.

After closing, decontamination efforts were made throughout the Widener Hospital. Rigorous, multistage cleaning and disinfection was performed which included four hospital wards, particularly for horses; a ward for large ruminants; an isolation facility; a sports medicine building; a treadmill facility; and all operating rooms and diagnostic areas including paths connecting such buildings. In many animal-housing areas, grounds and/or floors were sandblasted and/or resurfaced. Some flooring surfaces were completely removed and replaced with concrete plus a polyurethane-based monolithic flooring system, where appropriate. Flooring surfaces in stalls and other areas of the ICU/NICU facility were removed and replaced and not subject to sandblasting. Equipment and supplies were cleaned or discarded. Liquid disinfectants were applied on most surfaces throughout the hospital. Particular use was made of an aqueous solution containing potassium peroxomonosulfate, sodium dodecylbenzenesulfonate and sulfamic acid (Virkon-S®; Antec International, Sudbury, UK), an AOAC-approved oxidizing detergent sanitizer. While the bacterial population was successfully reduced at many locations, the population persisted within the ICU/NICU. After several failed attempts at controlling the Salmonella population within this facility, it was decided that a gas-phase space decontamination would be the most effective route to a successful decontamination.

Physical Site

The ICU/NICU comprises a nearly isolated building, adjoined through one hallway to an equine orthopedic ward (Figure 1). The total volume of space considered for decontamination is approximately 4800 m³ (170,000 ft³). Its main floor is comprised of fifteen patient units with wooden sliding doors and appropriate facilities for providing food and waste disposal. This floor also has two nursing stations, a laboratory, a pharmacy, three rest-rooms, several offices and other smaller rooms. Two weighing stations were built into the floor and an overhead hoist system is present to facilitate the movement of patients and equipment. A second floor contains additional offices, a restroom, kitchenette, and a mechanical room housing the exhaust and supply blowers. Two basement rooms house additional mechanical and storage space, and two elevators and an internal stairway adjoin the floors. Although much of the space had been disinfected, office rooms remained furnished and contained files, as well as other personal effects. Interstitial spaces were visibly soiled. The interstitial space above the basement is open to the earth foundation under part of the building. The HVAC (heating, ventilation and air conditioning) system is 100% in/out. Most of the offices have window air conditioners.

Decontaminant Choice

Three fumigants were considered for the space decontamination of the ICU/NICU—formaldehyde gas, hydrogen peroxide vapor, and chlorine dioxide gas. All three were known to be effective decontaminants for spore and non-spore forming bacteria under standard laboratory conditions, i.e., clean flat surfaces lacking porous materials or potential dead-legs with which fumigant
penetration might be retarded.

Standard application of formaldehyde (Taylor et al., 1969) typically involves the use of ammonia gas following the decontamination cycle to neutralize the remaining formaldehyde (Luftman, 2005). A residue consisting of polymerized formaldehyde (paraformaldehyde) and the neutralization product (methenamine) is commonly left after such treatment. The removal of such a residue was considered problematic for this facility. Residual formaldehyde from off-gassing was also of concern, due to its odor and its toxicity. Formaldehyde is considered a potential carcinogen by the EPA and an actual carcinogen by the International Agency for Research on Cancer (IARC, 2004).

Hydrogen peroxide vapor, when used as a space fumigant, is typically generated at a concentration that is close to or above its condensation point under typical temperature and humidity conditions (Watling et al., 2002). Different systems attempt to either prevent or promote this condensation. Nevertheless, as a result of this physical instability, there is a need to have rapid and very efficient recirculation of the hydrogen peroxide if one is trying to prevent condensation, or to inject the vapor at nearly line-of-sight to all surfaces if condensation is desired. These conditions were believed to be too restrictive for the current application, particularly when all interstitial space and ductwork were to be included within the decontamination. Furthermore, hydrogen peroxide is known to be chemically unstable in the presence of cellulose-based material, and the ICU/NICU space contained many wooden surfaces within the clinical areas and paper within the offices. It was believed that it would have been difficult to maintain an appropriate concentration of active hydrogen peroxide within the space.

The performance of disinfection by chlorine dioxide (Knapp & Battisti, 2001; & Knapp et al., 1986) had recently gained attention by virtue of its application against anthrax spore contaminations at the Hart Senate Building and the Brentwood Mail Facility in Washington, DC and of the U.S. Postal Service Trenton Mail Processing and Distribution Center in Hamilton, New Jersey (Haas, 2001; & U.S. EPA, 2005). It has also been employed with sterilizers, isolators, and small rooms (Leo et al., 2005). Chlorine dioxide (ClO₂) is a water soluble, yellow-green gas with a boiling point of 10°C. Dissolved in water, it has been used as a germicide in water and food treatment and as a bleaching agent by the paper industry. ClO₂ is a selective oxidant reacting primarily with organics that are highly reduced (e.g., alcohols, aldehydes, ketones, tertiary amines and sulfur-containing amino acids) and thus is generally not as adversely affected by typical organic loads, as are other oxidants, such as hydrogen peroxide (Knapp & Battisti, 2001). Its deleterious effect on bacterial endospores is believed to be directed primarily toward the cell membrane rather than DNA (Young & Setlow, 2003). Unlike bleach or chlorine gas, ClO₂ is known not to form chlorinated by-products (Knapp & Battisti, 2001). As a selective oxidant, it has also been shown to be
compatible with most standard materials including stainless steel, anodized aluminum, Teflon, Viton, polyethylene, polypropylene, and nylon (Eylath et al., 2003; Kowalski, 1998; & Leo et al., 2005). Some discoloration of uncoated copper and cold roll steel had been observed, comparable to what is seen when those materials are exposed to high humidity environments.

Several issues needed be addressed in planning for this method of decontamination. As with typical sporicidal fumigation protocol, the space would require sealing in order to maintain a sufficiently high concentration of the fumigant for efficacy, as well as to maintain a safe perimeter outside the building. A relative humidity greater than 60% would need to be maintained within the space for optimal ClO₂ potency. ClO₂ would need to be generated on site due to its short-term chemical instability. Because chlorine gas (Cl₂), is highly corrosive to many materials, the method of preparation would need to ensure its absence from being present within the ClO₂ flow. Furthermore, because ClO₂ can dissociate releasing Cl₂ in the presence of ultraviolet light, ultraviolet sources such as sunlight need to be avoided.

Method and Materials

Site Preparation

Sealing the facility proved to be the most time-consuming, labor-intensive part of the project. In part, this was because the ICU/NICU building had not been designed with the intent of ever requiring fumigation. Most of the sealing was performed on exterior surfaces. All vents or potentially leaky seams along the roof, which included vents adjacent to roof gutters and ridge vents, were sealed using combinations of caulk, expanding foam, duct tape and adhesive films. The exhaust and air supply units for the building were deactivated and their associated vents on the exterior walls and roof were sealed. External switches for these units were supplied to enable reenergizing them following the decontamination without having to enter the gas-filled space. Office windows that were capable of being opened were sealed, as were office air conditioning units. Obvious external wall damage and cracks were sealed. Vents for the two basement rooms were sealed. All external doors were ultimately sealed, as was an internal door leading from the ICU/NICU building to the adjacent 17-stall orthopedic ward. Several cover plates and plumbing fixtures on the outer walls and floors were sealed. The two dump holes on the underside of the building were sealed.

Aside from the door leading to the adjacent barn, all internal doors were propped open. All drawers and cabinet doors were opened and light diffusers were removed. All access panels to interstitial spaces were opened within the main and second floors. Access panels within the basement rooms were sealed, because the adjacent interstitial space opened to an earth foundation and was considered not capable of being disinfected and a potential source of loss of the fumigant by absorption or reaction. Internal supply and exhaust vents were left open. Water was poured into all floor and sink drains that had traps, while other drains were capped.

Over 40 fans and blowers were distributed throughout the facility to ensure ClO₂ circulation. Several of these were placed at open access panels, within ceiling interstitial regions. A blower, connected by flexible duct, was inserted between openings within the main building exhaust and return units to force circulation throughout the HVAC system. The blower within the one biological safety cabinet in the building was turned on during the decontamination.

The efficacy of ClO₂ as a decontaminant is greatly enhanced at a relative humidity greater than 60% (Jeng & Woodworth, 1990). Thirty-five pans on hot plates were placed throughout the facility, with the intention of boiling water just before decontamination. Gauges to measure relative humidity and temperature were placed at several windows, allowing the internal conditions to be monitored during the decontamination.

A test of the containment of the ICU/NICU was performed. All circulating fans were energized. All except one of the entrances to the building were sealed. Three pans with 200 g isomyl acetate and approximately 100 ml water were placed upon hotplates within the ICU/NICU and the hot plates were energized. Leaking sites were then sought along all external surfaces, including the roof and basement areas of the ICU/NICU, by scenting for the isomyl acetate. One major leak at the wall adjoining the ICU/NICU to the adjacent 17-stall equine ward and several minor leaks along the roof were detected. Specific leak sites were visualized using smoke generated by a glycol fogger, and subsequently repaired.

Biological indicators were aseptically placed a few hours before the decontamination throughout the building, including within ceiling interstitial spaces and the HVAC exhaust, to corroborate the efficacy of the decontamination (Figure 2). The indicators included (a) 40 unwrapped strips each with approximately 2 x 10⁶ spores of G. stearothermophilus (Spordex), which are considered to be the most difficult challenge for ClO₂ decontamination (Leighton et al., 2004); (b) 20 unwrapped spore strips of B. atrophaeus (Spordex), previously known as Bacillus subtilis var. niger; and (c) 40 strips inoculated with approximately 10⁹ colony forming units (CFU) of the bacterium Salmonella Newport. [The S. Newport test strips were prepared by the PADLS Salmonella Reference Center (SRC). They consisted of 1 x 1.5 inch absorbent paper with an impermeable plastic backing (Benchkote™), inoculated with 1 ml of a 10⁶ ml⁻¹ suspension of the bacteria. The strain of Salmonella had previously been isolated from an environmental sample submitted to the PADLS laborato-
ries for culture. A representative batch of the test strips was tested for long-term viability prior to use. The culture work was performed in the PADLS/UP Clinical Microbiology laboratories and the serogrouping and serotyping was undertaken by the SRC.]

It was intended that the actual decontamination take place during nighttime hours, primarily to minimize the amount of photo-dissociation that is known to occur with ClO₂. Circuit breakers for all emergency lighting within the ICU/NICU were identified so that all internal lighting would be off during the decontamination. Pressure manometers with tubes extending under external doors into the building were placed at four locations about the ICU/NICU to ensure that the differential pressure from outside to inside the building remained as close to neutral as possible throughout the decontamination.

**Chlorine Dioxide Preparation**

Chlorine dioxide was to be generated from chlorine gas and sodium chlorite by the reaction:

\[
\text{Cl}_2 (g) + 2\text{NaClO}_2 (s) \rightarrow 2\text{ClO}_2 (g) + 2\text{NaCl} (s)
\]

2% Cl₂ gas in nitrogen was passed through columns packed with solid sodium chlorite and other stabilizing material. Columns were appropriately sized such that 100
g Cl\textsubscript{2} was fully consumed by the above reaction with no measurable (<1 ppm) Cl\textsubscript{2} exiting from the column. Ten such generative systems were placed outside the ICU/NICU, with delivery tubes leading to various locations within the site. Several delivery tube lines were branched within the facility to further enhance gas distribution. Real-time concentration analysis of the ClO\textsubscript{2} was performed by ultraviolet/visible absorption measurements near the peak absorbance for ClO\textsubscript{2}. A spectrophotometer (ClorDiSys Solutions, Inc.) was situated outside the ICU/NICU with 10 tubes for gas sampling running from the spectrophotometer to various locations within the ICU/NICU. A sampling manifold was utilized to switch detection from one area to another.

The intent was to attain a concentration of 1 mg ClO\textsubscript{2} per liter of air within the ICU/NICU after 4 hours of delivery, followed by an additional 1 hour of contact time at the targeted level, and then aeration. Prior experience by ClorDiSys Systems, Inc. indicated that the overall dose would be capable of delivering a 8-log kill to spores of B. atrophaeus, which is substantially more resistant to decontamination than gram-negative vegetative bacteria, such as S. Newport.

The Decontamination Event

The generation and introduction of the ClO\textsubscript{2} was initiated at 9:00 p.m. Exterior temperature at the time was approximately 75°F with a relative humidity of 70%. Due to the high humidity, the use of the hot plates for further humidification became unnecessary. The intended schedule consisted of building up gas concentration to a level of 1 mg ClO\textsubscript{2} per liter of air (~350 ppm) until 1:00 a.m., maintaining same concentration until 3:00 a.m., and then aerating until 9:00 a.m. The intent was to have a total exposure (the product of concentration and time) of 3.0 mg (ClO\textsubscript{2})–hour/liter (air) or 1050 ppm-hours. It was estimated that the exhaust capability of the ICU/NICU was on the order of 170 m\textsuperscript{3}/min (6000 ft\textsuperscript{3}/min) and the building volume was approximately 4800 m\textsuperscript{3} (170,000 ft\textsuperscript{3}). A 6-hour aeration was estimated to bring the concentration of ClO\textsubscript{2} from its target level of 350 ppm to safely below its PEL limit of 0.1 ppm.

Internal temperature, relative humidity and pressure differential to the outside were monitored at the beginning and during the decontamination. Values were acceptable throughout, with the exception of one low relative humidity reading, later attributed to the meter being incorrectly set. There was no measurable amount of ClO\textsubscript{2} detected outside the decontamination area, although the gas could be smelled within the barn in the area adjacent to the sealed ICU/NICU. A fan was placed within this area to prevent gas accumulation. The concentration of ClO\textsubscript{2} was monitored serially from 10 locations regularly during the treatment. The spectrophotometer was sensitive to approximately 0.1 mg ClO\textsubscript{2}/liter air. The raw measurements are shown in Table 1.

After 2 hours, it was ascertained that the concentration of ClO\textsubscript{2} was not increasing at the anticipated rate. Previously sealed points about the ICU/NICU were again investigated. A minor amount of ClO\textsubscript{2} leakage was detected and immediately sealed at points along the roof and at plumbing fixtures on the eastern wall. Nevertheless, the level of ClO\textsubscript{2} still did not increase at the desired rate and it was evident that the target concentration level would not be attained. It was decided to modify the schedule by continuing the introduction of ClO\textsubscript{2} until 8:00 a.m. (660 min), allowing for an overall increase of ClO\textsubscript{2} exposure. The end time was set, in part, to minimize the effect of photo-disassociation of the ClO\textsubscript{2} from sunlight. The total integrated doses of ClO\textsubscript{2}, calculated from the data in Table 1, are shown in Table 2.

The average ClO\textsubscript{2} dose of 1.13 mg-hour/liter air (or

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Concentration Measurements of ClO\textsubscript{2} (mg/liter air).</td>
</tr>
<tr>
<td>Sample Site #</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
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<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>
~400 ppm-hr) was 38% that of the target level of 3.0 mg-hour/liter air. It was estimated at the time that this exposure would be sufficient for at least a 5-log population reduction for the spore strips and a greater reduction for the S. Newport strips.

**Chlorine Dioxide Aeration**

Personnel wearing self-contained breathing apparatuses commenced aeration that morning and the sealing materials from exhaust and intake fans on the roof were removed and the fans were energized. A whitish emission was seen rising from the vent points and a chlorine odor was detected at ground level north of the ICU/NICU, ceasing within 50 minutes. Dräger measurements in these vicinities indicated that the level of ClO$_2$ and/or Cl$_2$ remained below the detection limit of 0.1 ppm. An external door was opened after an hour to aid with ventilation. The concentration of ClO$_2$ within the ICU/NICU was monitored periodically during the following hour. At the onset of aeration, the interior space was foggy and ClO$_2$ levels up to 10 ppm were measured. After 2.5 hours, the level of ClO$_2$ was no longer measurable and the space could be entered without personnel protective equipment. The reduction of required aeration time from the anticipated 6 hours was consistent with the lower than planned concentration of ClO$_2$ at the onset of the aeration.

**Biological Indicator Analysis**

Biological indicators were then aseptically collected throughout the ICU/NICU. All of the exposed spore strips impregnated with Geobacillus stearothermophilus and Bacillus atrophaeus and two unexposed strips were delivered to Microbiological Environments for determination of remaining viability. The two unexposed indicators served as controls to verify recovery efficacy and to calculate the logarithmic population reduction. The strips of S. Newport were delivered to the PADLS (University of Pennsylvania Clinical Microbiology Laboratories) for culture.

The B. subtilis and G. stearothermophilus biological indicators were processed the same day they had been received. Determination of the viable spore population was accomplished by aseptically transferring each spore strip to 30 ml of a sterile Butterfield’s buffer solution. A cell disruptor was used to completely macerate the paper carriers and 10 ml aliquots were plated. The control samples were diluted 10-fold to the predicted population and 1 ml aliquots were plated. The plates were incubated under growth-permissive conditions.

Forty-two S. Newport test strips (including one unexposed control and one uninoculated strip) were each placed in 100 ml of Buffered Peptone Water (BPW) Oxoid CM0509 (Oxoid; Ogdensburg, NY). The BPW cultures were incubated at 37ºC for 18 hours. Following incubation, 0.1 ml aliquots of BPW were transferred to 10 ml of Rappaport Vassiliadis (RV) Oxoid CM0669 enrichment medium. The RV broths were incubated at 42ºC for 18 hours. After incubation, the RV cultures were each sub-cultured on McConkey’s Agar (MC) Oxoid CM507, Difco XLD medium (XLD) and Desoxycholate-Citrate Agar (DCA) Oxoid CM0227.

**Results**

The plate counts for the two Geobacillus stearothermophilus control strips, each diluted by a factor of 3.0 x 10$^6$, were 16 and 27 colony forming units (CFU). These correspond to viable spore populations of 4.8 x 10$^5$ and 8.1 x 10$^5$. The mean value of 6.5 x 10$^5$ spores was used as the

**Table 2**

<table>
<thead>
<tr>
<th>Site #</th>
<th>Location</th>
<th>Dose (mg ClO$_2$—hour/liter air)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Main Floor, east end</td>
<td>1.44</td>
</tr>
<tr>
<td>2</td>
<td>Main Floor, west end</td>
<td>1.13</td>
</tr>
<tr>
<td>3</td>
<td>Room 154</td>
<td>0.63</td>
</tr>
<tr>
<td>4</td>
<td>Room 157</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>Loft stairwell</td>
<td>1.93</td>
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<tr>
<td>6</td>
<td>Office 201</td>
<td>1.73</td>
</tr>
<tr>
<td>7</td>
<td>Loft mechanical room</td>
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<tr>
<td>8</td>
<td>HVAC return</td>
<td>0.46</td>
</tr>
<tr>
<td>9</td>
<td>Office 144</td>
<td>1.28</td>
</tr>
<tr>
<td>10</td>
<td>Eastern elevator, basement</td>
<td>1.01</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.13</td>
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reference for reduction calculations. Of the 40 plates produced from the G. stearothermophilus biological indicators placed within the ICU/NICU before the ClO₂ decontamination, 32 yielded no growth, seven yielded one CFU, and one yielded two CFU. There was no discernible pattern as to the location of the biological indicators that corresponded to non-zero CFU plates.attributing no growth to an upper limit of 0.9 CFU and given a dilution factor of 3 for the test plate studies, no growth, 1 and 2 CFU correspond to log reduction values of >5.5, 5.4, and 5.1, respectively.

The plate counts for the two Bacillus atrophaeus control measurements, each diluted by a factor of 3.0 x 10⁶, were 109 and 112 CFU. These correspond to viable spore populations of 3.27 x 10⁶ and 3.36 x 10⁶, respectively. The mean value of 3.32 x 10⁶ spores was used as the reference for reduction calculations. Of the 20 plates produced from the B. atrophaeus biological indicators placed within the ICU/NICU before the ClO₂ decontamination, all 20 yielded no growth. Attributing no growth to an upper limit of 0.9 CFU and given a dilution factor of 3 for the test plate studies, no growth corresponds to log reduction values of ≥6.14.

Of the 40 Salmonella test strips processed, S. Newport was recovered from 2, with the remaining 38 testing negative for the presence of Salmonella. The inoculated but unexposed strip was positive for S. Newport and the uninoculated control strip was negative. No attempt was made to perform viable counts in order to calculate log reductions.

Within 3 hours following the onset of the aeration of the ICU/NICU from ClO₂, no residual odors within the ICU/NICU were detected. Furthermore, the pre-existing "animal" odors were also absent. No physical residue corresponding to the ClO₂ was observed and there was also no visible indication of material degradation—specifically, no obvious indication of corrosion of any of the metal-containing equipment that had been left within the ICU/NICU. Mechanical and electrical items that had been present within the ICU/NICU during the decontamination all continue to be functional at this time.

After receipt of the analytical results from the biological indicators, sealing material was removed from the ICU/NICU one week following the decontamination event. Six sets of hand and foot area Swiffer® tests for Salmonella (noted in the introduction) were taken throughout the ICU/NICU during the four months following the decontamination. A typical sample set included hand and foot samples for the nursing station (encompassing the two nursing stations, laboratory and pharmacy); main floor of the ICU; stall composite for the ICU; main floor of the NICU; stall composite for the NICU; and the link corridor. Hand and floor samples composited from areas like bathrooms, offices, the upper floor and the basement were included on a rotating basis. All results were negative. Following decontamination, the flooring of the ICU/NICU nursing stations, laboratory, pharmacy and those in the muck rooms and feed storage areas were replaced with a poured epoxy surface that was coved up the walls. A monolithic polyurethane-based equine flooring system was installed in each of the stalls. Flooring that had been present in these areas (tile and rubber mats) had all been removed before the decontamination event. In addition, casework and plumbing fixtures, most of which had been removed before decontamination, were reinstalled. Automatic waterers formerly present in each stall were not replaced, but repairs to the block walls and repainting were undertaken before reopening. Ceiling tiles which had been removed in preparation for the ClO₂ decontamination were replaced, and damaged dry-wall ceiling or wall areas due to the liquid disinfection phases were repaired and repainted. New laundry facilities were also put in place; modifications and repairs to electrical and telephone systems were made and the entire area was restocked. Immediately prior to reopening, the air handling system of the building was rebalanced and culture sets taken both before and after air balancing were all found to be negative for Salmonella. Although the main part of the hospital initially reopened for cases on August 2, 2004, and the office spaces within the ICU/NICU were back in use shortly after decontamination, the extensive remediation and remodeling efforts undertaken in the clinical and animal housing areas of the building delayed facility reopening to patients. The building was, however, declared fully operational as of January 18, 2005. Since reopening and starting to accept patients, weekly environmental sample sets have been collected. At the time of writing, all samples have been negative for Salmonella.

Discussion

The targeted concentration and dosage of ClO₂ used for the ICU/NICU decontamination were based upon prior experience of two of the authors (Lorheim and Czarneski) and upon studies within the literature. Substantially greater concentration and dosages have been used in other reported work. Han et al., 2003 experimented with initial concentrations of 1500 to 10,000 ppm for up to 12 hours exposure, concluding that if the ClO₂ were not replenished, the higher limit of concentration might be required. The Environmental Technology Verification study of ClO₂ (Rogers et al., 2004) employed a relatively constant concentration of 2000 ppm with a 6-hour exposure. Based upon studies of the anthrax spore remediation in Washington, DC, the U.S. EPA issued a crisis exemption for the use of ClO₂ at a total dosage of 9000 ppm-hr. (U.S. EPA, 2005). Leighton et al. (2004) estimated spore survival probabilities of well below one out of a million from laboratory studies using strips with
10^6 spores of *B. atrophaeus* and *G. stearothermophilus* with four-hour exposures of ClO\textsubscript{2} at 500 ppm. Their statistical modeling indicated that the survival fraction for *G. stearothermophilus* spores would be below 5 x 10^6 for biological indicators with 10^6 spores with a dose of 500 ppm-hr at 80% relative humidity. Jeng and Woodworth (1990) demonstrated a D value (the amount of time required for a 90% or 1-log reduction in viability) for *B. atrophaeus* of 1.6 min using 2500 ppm ClO\textsubscript{2} with 10^6 spores deactivated within 15 min exposure. Leo et al. (2005) demonstrated 4-log reduction of *B. atrophaeus* after a 30 min exposure to 1.0 mg/l and a similar reduction in 10 min with 3.0 mg/l ClO\textsubscript{2} (1000 ppm). Lorcheme (Kowalski, 1998) used unwrapped paper carriers impregnated with *B. atrophaeus* spores to obtain D values of 4.9 and 3.6 min. with ClO\textsubscript{2} concentrations of 3 and 5 mg/l (1000 and 1600 ppm), respectively. The targeted level of ClO\textsubscript{2} exposure for this project, approximately 1000 ppm-hr. for an 8-log kill, had been based largely upon these latter data.

With the actual exposure of ClO\textsubscript{2} in this work at approximately 400 ppm-hr., we anticipated a log reduction of *B. atrophaeus* spores to be on the order of 5 to 6, a level borne out by the results. A significantly greater population reduction was anticipated for the species of concern, *S. Newport*, as bacterial spores are known to be substantially more resistant to the gas than are vegetative cells (Knapp & Battisti, 2001).

As previously noted, the concentration of ClO\textsubscript{2} within the ICU/NICU did not increase at the anticipated rate given the rate that Cl\textsubscript{2} was introduced into the ClO\textsubscript{2} reactors. Furthermore, as may be inferred from Table 1, the concentration of ClO\textsubscript{2} within the ICU/NICU decreased at a significant rate when the Cl\textsubscript{2} flow was interrupted. Three potential causes for this loss have been considered: 1) leakage of ClO\textsubscript{2} to the ambient; 2) absorption of ClO\textsubscript{2} by various materials within the ICU/NICU; and/or 3) decomposition of ClO\textsubscript{2} within the ICU/NICU by interaction with materials or other environmental factors, such as ultraviolet light. Major leakage sites were found and remediated before the decontamination. Minor leakage sites detected during the initial hours of the decontamination were remediated as discovered. While some ClO\textsubscript{2} continued to be sensed outside of the space near the junction between the ICU/NICU and its adjoining building during the decontamination, the local level did not exceed the Dräger tube sensitivity of 0.1 ppm. The mean concentration within the ICU/NICU during the decontamination was approximately 40 ppm. Thus, leakage does not appear to have been significant enough to account for much of the loss.

Accelerated decomposition of ClO\textsubscript{2} would be anticipated to occur either through reductive reactions or photolysis. Light-induced decomposition can be ruled out as a significant source of gas loss as the process was performed during the night and there were no lights energized within the space. As noted previously, ClO\textsubscript{2} is a fairly selective oxidant, which is not expected to react with typical building materials (including wood) or soil. Buttner et al. (2004) noted that a soiling load had no notable effect on the efficacy of ClO\textsubscript{2} on *B. atrophaeus* endospores, although they did not report whether more gas generation was required to maintain their target concentration. Han et al. (2003) noted significant loss of ClO\textsubscript{2} during experiments with various materials within a sealed 10-liter vessel, which the authors attributed to decomposition or absorption. As noted previously, there was a substantial soil load within the ICU. The decontamination space encompassed two elevator shafts, ductwork, mechanical spaces and interstitial spaces that had not been previously cleaned. Interstitial space associated with the basement was contiguous to a soil foundation. While an attempt was made to isolate this particular space from the decontamination, it was imperfect. The space encompassed multiple offices containing file cabinets and desks containing paper, cardboard, and personal items. ClO\textsubscript{2} would also preferentially dissolve into open water sources associated with restrooms within the space. Future experiments within controlled spaces are in order to isolate the most significant absorption and/or reaction sites to aid in the practical design of decontaminations in the field.

Independent of the cause for the loss of ClO\textsubscript{2}, the importance of being able to monitor the concentration of the gas in real time was accentuated, particularly for spaces having such a variety of potential issues. Flexibility in the decontamination, such that modification of the amount of introduced decontaminant managed, would also be useful.

**Conclusion**

Large animal intensive care and neonatal intensive care units were contaminated with *Salmonella* Newport, thereby resulting in the infection of multiple patients and the ultimate quarantining of the facility. Chlorine dioxide gas was used to decontaminate the 4800 m\textsuperscript{3} facility. After the facility had been appropriately sealed, a total ClO\textsubscript{2} dosage of ~400 ppm-hr. was applied over a single evening. The process was monitored with 100 biological indicator strips inoculated with *Geobacillus stearothermophilus* spores, *Bacillus atrophaeus* spores, or *Salmonella* Newport vegetative cells. Subsequent analysis indicated better than 6-log viable reductions for the *B. atrophaeus* and *S. Newport* and better than 5-log reductions for the *G. stearothermophilus*. No deleterious material effects were noted. The facility has been reopened and no similar infections have been reported to date. ClO\textsubscript{2} has been demonstrated to be an effective decontaminant under non-laboratory conditions.
References


When it comes to researching biohazards for the federal government, be careful what you wish for. The contracts are lucrative—the University of Washington has secured a $50-million grant from the National Institutes of Health (NIH) and has applied for another worth $25 million—but the money comes with tradeoffs and compromises that university researchers and administrators need to consider carefully. Even cutting-edge facilities might require upgrades, security doubtlessly will require costly enhancements, and everyone involved will need to accept a significant culture change that can be uncomfortable for those accustomed to working in the open atmosphere of university research.

“The bottom line is, if you accept the money you need to adhere to all the regulations associated with it,” says Stephanie Steppe, director of health science academic services and facilities at the University of Washington. “You need to think about those things before you ever apply for the grant.”

Three years ago, the University of Washington applied to host the WWAMI Regional Center of Excellence (RCE) for Biodefense & Emerging Infectious Diseases Research, one of only eight in the country and the only one west of the Mississippi River. WWAMI refers to the five-state consortium including Washington, Wyoming, Alaska, Montana, and Idaho.

An RCE pulls together researchers who are working in emerging infectious diseases. Institutions draft joint operating agreements and submit a grant application to become an RCE. The University of Washington has joined forces with the universities of Montana, Idaho, and Alaska at Fairbanks; Seattle’s Fred Hutchinson Cancer Research Center, Harborview Medical Center; the Institute for Systems Biology; the Veteran’s Administration in Puget Sound; and the Rocky Mountain Laboratories in Montana, run by the NIH’s National Institute of Allergy and Infectious Diseases.

“We created a think tank,” says Steppe. “We each do research in our local labs and come together at least quarterly to exchange information.

“Many university faculty are doing research in a silo,” she says. “They will have their own staff; they will have their own research expertise. When you are talking about Regional Centers, you need to think about collaboration versus competition.”

In order to conduct key RCE research, a laboratory must meet federal qualifications to handle select agents, which are defined by the Centers for Disease Control’s Select Agent Program as “biologic agents and toxins that pose a severe threat to public health and safety.”

“We became an RCE two years ago, and the impact to the University of Washington by accepting the $50-million grant is extensive,” says Steppe.

**Substantial Upfront Investment**

The University of Washington receives more federal grants and contracts than any other public institution in the United States, says Steppe, but even they were surprised by the process involved in becoming an RCE.

The first step in deciding whether or not to pursue one of these grants was to assess the state of their lab facilities. The University of Washington already has several separate BSL-3 laboratories, so it did not need to build new ones for the RCE. Steppe manages what she calls the “adventure” of a building containing three million gsf of space, with 13 separate fire zones. Of that, 7,000 asf are now designated for the RCE, including three BSL-3 labs and three BSL-2 labs, two animal labs, and various support spaces.

“There were researchers in those laboratories already that were not part of the RCE,” she says. “We had to shift researchers around which was not particularly pleasant, but we got through it.”

It is also important to understand the level of administrative support this kind of research requires, cautions Steppe.

The University first organized a team to prepare the application. That team met almost weekly for three years to go over all the critical issues related to the RCE, first regarding the application, then implementing the grant, and eventually administering the RCE itself.
“Conservatively, we have spent $250,000 worth of salary not paid by the grant itself in order to make this program work,” says Steppe. “Can your institution afford that?”

A large part of the administration is focused on complying with the complex laws regulating the biohazard industry, including the Anti-terrorism and Effective Death Penalty Act of 1996; the USA Patriot Act of 2001; the Public Health Security and Bioterrorism Preparedness Response Act of 2002; and, most significantly, 42CFR Parts 72 and 73—Possession, Use, and Transfer of Select Agents and Toxins.

Keeping an eye on all this is the designated Responsible Official (RO). In the case of the University of Washington, it is the director of environmental health and safety, who works with two alternates to assure compliance at all University facilities. Other institutions might appoint other individuals, such as the vice president of research, to fill this critical role. How that position is filled depends on the size and configuration of the institution, says Steppe.

“These ROs definitely take their job seriously,” she says. “They personally face a potential $250,000 fine and five years in prison” for willful oversights and violations.

The RCE research process also must involve a concerted community outreach campaign. Steppe advises institutions to begin that campaign soon after applying for the grant, even before the application is funded.

“Responding to the community after the fact can be difficult and embarrassing for the institution, if the community feels the institution is keeping secrets. This gap in time allows a lot of unwarranted fears to come forth from the community,” she says.

Satisfying Everyone

One frustration for the University has been determining appropriate processes to meet the expectations of many agencies with differing jurisdictions, such as the NIH, the Office of the Inspector General, the CDC, the FBI, the Department of Justice, and the U.S. Attorney General’s Office. To aid in complying with these various issues, the University hired a consultant who specializes in certification of similar facilities. Many renovation costs were generated directly by the findings of the certifying agent, which was not literally true, but we requested modification to the HVAC system to satisfy that concern,” says Steppe.

“Then the CDC inspectors told us to replace all the wooden casework with metal casework, but I found out later at a Tradeline conference that the CDC uses wooden casework in their own BSL-3 labs,” she says. “The certifying agent also told us to take out the ping-pong indicators we used as a secondary visual backup for negative air pressure, and it turns out that the ones we are removing are the same model that the CDC uses.”

Steppe cautions institutions to weigh the decision about whether to hire a certifying agent or a commissioning agent. A certifying agent, who is normally less expensive to hire, will inspect a facility and point out a facility’s inadequacies, but might not provide the detailed design necessary to rectify all the problems. A commissioning agent, on the other hand, might be worth the added expense because that company will help redesign or resolve any issues that arise during renovation and inspections.

Culture Shock

Security regulations regarding select agents can cause the most upheaval in an institution. The University of Washington already employed proximity card access and photo IDs in many of their facilities. Following a security assessment conducted by the CDC and the chief of the University’s own police department, the system was enhanced with the addition of biometrics.

Every person working in a facility containing select agents—from the primary investigators to the custodial staff—must significantly modify their work activities.

The University used to be free to hire whomever it found qualified. Now anyone who has unsupervised access to the RCE must undergo an extensive FBI risk assessment and background check. Steppe says it took her four months to get clearance, while others may take as long as six months. Some people may never gain that access based solely on their country of origin, as stipulated by the USA Patriot Act, even if they have worked for the University for years.

“Universities have been known to return federal dollars because they do not want to deal with some of the aspects of the USA Patriot Act,” says Steppe. “It could limit careers of individuals who might have come to your university from one of the seven countries listed in the Act.”

Human resources must also be involved in developing procedures for these new employee questions and records that contain information about whether an individual is a convicted felon, has ever been dishonorably discharged from the military, or was institutionalized for mental reasons.

“Under current personnel guidelines, the supervisor
cannot know this information about their employee, so it cannot be kept in the regular human resources file," explains Steppe. "We have had to develop an entirely new process for that paperwork, who has access to it, and who physically stores it."

Once hired, researchers need to be vigilant about following procedures for monitoring access privileges and handling select agents.

"Our faculty is having difficulty adjusting to that," says Steppe. "A university is all about free ideas, free access, and free movement. The ones who are part of the RCE recognize that they have to think about how they move through the corridors, who they talk to, and what they talk to them about. They are not working in secret, yet normal exchange of intellectual property is happening in a different environment. Some people buy into it; some people do not."

Administrators face additional paperwork and the same atmosphere of reticence. For example, building operating procedures that used to be kept only by the facilities managers must now be duplicated and kept in the RCE labs themselves.

The maintenance staff who will work in buildings containing select agents also must alter their procedures.

"They don’t get to open the door with their set of grand master keys, walk in, change the light bulb, and walk out," says Steppe.

Obtaining FBI clearance for all maintenance, alterations, and custodial staff is not feasible, says Steppe. Instead, it makes more sense to establish procedures for escorting these critical workers whenever they need to be in the RCE. They are never left alone, and they never work after hours unless they are accompanied by an authorized individual. Guests and vendors must be escorted, as well, if they are to be in the labs at all.

You Never Work Alone

This heightened level of activity within the facility extends into the greater community. In addition to the police, the university must also ensure that they have a strong relationship with the local fire department and emergency medical personnel, while balancing the need for discretion with the need to share vital information.

"That is a conflict we continue to address each day as we interpret and reinterpret all of the regulations," says Steppe. "In the event of an emergency or release, there are a lot of people who are going to get involved very quickly."

That list includes the local emergency rooms.

"You don’t just walk into your local ER and say, ‘Hi, I’m from the Regional Center of Excellence. We are doing research with pestis,’ and expect the emergency personnel to know what to do with an individual who has been exposed to pestis."

Undaunted by this process, the University of Washington has applied for a construction grant to build a Regional Biocontainment Laboratory (RBL), which would add further research and development components to the existing allergy and infectious diseases program. The NIH funds the construction of RBLs (with BSL-2 and BSL-3 capabilities), and National Biocontainment Laboratories (with BSL-4s) for research and training in biodefense and emerging infectious diseases. If approved, the project will require the University to build a $64-million facility with a $25-million NIH grant, $8.3 million from the University, and the rest from other sources. The University expects to hear in September if it has been approved for the grant.

Biography

Stephanie Steppe is director of health science academic services and facilities at the University of Washington, where she directs a staff of 90. Her department provides infrastructure and support services for 8,000 health sciences staff and students in three million gsf. Her group service menu consists of building management, classroom services, client services, clinical skills, creative services, educational technology services, laboratory services, finance and personnel, scientific instruments, and technology services.

This article is based on a presentation given by Stephanie Steppe at Tradeline’s International Conference on Biocontainment Facilities in April 2005.

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### EPA Pesticide Program Update from EPA’s Office of Pesticide Programs

**10/11/05: EPA Approves New Non-Chemical Control for Corn Rootworm**

www.epa.gov/pesticides

After an intensive, multi-year scientific analysis, EPA has approved applications submitted by Mycogen Seeds (c/o Dow AgroSciences, LLC) and Pioneer Hi-Bred International, Inc. for the use of a new corn plant-incorporated protectant (PIP) designed to control corn rootworm. Corn rootworm is a widespread and destructive insect pest responsible for the single largest use of conventional insecticides in the United States. The new product is the second PIP to offer protection against corn rootworm and is expected to result in a further reduction of chemical insecticide use by growers.

The new corn plant-incorporated protectant, Event DAS-59122-7 Corn, produces its own insecticide within the corn plant derived from Bacillus thuringiensis (Bt), a naturally occurring soil bacterium. The Bt proteins used in this product, called Cry34Ab1 and Cry35Ab1 (Cry 34/35), control corn rootworm.

To reduce the likelihood of corn rootworm developing resistance to Bt, EPA is requiring Mycogen and Pioneer to ensure that buffer zones within the planted acreage be planted with corn that is not protected from corn rootworm to serve as a “refuge.” The insect populations in the refuges will help prevent resistance development when they cross-breed with insects in the Bt fields. This resistance management strategy was developed as a condition of the registration, and EPA will require routine monitoring and documentation that these measures are followed.

The reduction in chemical pesticide use will benefit the environment directly and can mean less chemical exposure to people who apply pesticides to corn. The availability of multiple corn rootworm-protected corn products will also increase grower choice and price competition, resulting in lower seed prices for consumers and higher adoption rates. The product provides yet another way to combat corn rootworm, as well as indirect benefits such as energy savings resulting from reduced chemical insecticide use. As with similar products, EPA has approved Cry 34/35 for time-limited use, which will be subject to reevaluation in five years. For more information on EPA’s regulation of biopesticide products, see www/epa.gov/pesticides/biopesticides/.
USAMRIID Opens Advanced ABSL-3 Aerobiology Suite: Illustrates New and Unique Features for Animal Biosafety

Tradeline Publications

Orinda, California

The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) recently opened its newly renovated aerobiology ABSL-3 suite at Fort Detrick in Frederick, Maryland. The 4,800-sf facility includes labs and animal holding rooms designed for high throughput animal bioaerosol efficacy studies conducted with Select Agents and toxins.

The project, completed in September of 2005, involved the demolition of existing BSL-2 lab space and renovation of the space into a new ABSL-3 lab, including innovative animal containment and testing equipment that was custom-designed for USAMRIID.

The renovation project also included replacement of the air handling unit and mechanical and electrical utilities; installation of redundant HEPA filtration, exhaust fans, and associated ductwork; upgrade of the fire alarm system and building steam capacity; installation of a reverse osmosis water purification system and clean steam generator for room air humidification; and upgrades to physical and electronic security components to meet the Select Agents rule.

USAMRIID researchers conduct animal studies to develop and test biodefense vaccines, drugs, and diagnostics for U.S. military personnel. Within USAMRIID is the Center of Aerobiological Sciences, which conducts and supports research on pathogenesis, prevention, therapy, or treatment of disease and intoxications or poisonings, caused by exposures to aerosols of biological threat agents through the respiratory tract to the lungs.

All testing is conducted in accordance with Food and Drug Administration Animal Rule 21 (Code of Federal Regulations, CFR) parts 3.14 and 6.01, which allows for testing to be done on animals because of ethics considerations or because of the lethal nature of the materials being tested.

Catherine Wilhelmsen, DVM, PhD, CBSP, a microbiologist for USAMRIID, who also serves as USAMRIID’s Biological Safety Officer, points to the following design objectives as the overriding focus of the renovation project:

- Ensure safe, humane movement of animals.
- Optimize aerosol productivity and efficiency.
- Incorporate hands-free door operations.
- Stabilize the laboratory ambient temperature to prevent temperature fluctuations.
- Shorten sterilization time for soiled cages exiting the ABSL-3 facility.
- Provide flexibility for future changes in mission.

Ensure Safe Transporting

“Secure and effective animal transporting is our top priority so that we can guarantee safe conditions for both animals and researchers,” says Wilhelmsen. “With this in mind, we worked with Germfree Laboratories Inc. of Ormond Beach, Fla., to custom design mobile Class III-BSL animal transporters, the first such units to be used within USAMRIID.”

Six mobile transporters provide HEPA-filtered ventilated containment for rodents, rabbits, and non-human primates. Each battery-powered rechargeable transporter is equipped with its own power pack so it can move easily between the animal holding rooms and the three free-standing stationary Class III biosafety cabinets in the lab.

A rapid transfer (alpha-beta) port on each mobile transporter allows animals to be moved directly into identical ports on the biosafety cabinets. Once the ports are docked together, lab personnel must rotate and lock the handles in place before the doors can be opened to complete the animal transfer. The transporter also docks directly to a custom-designed port within the doors of each of the four BSL-3 animal holding rooms.

The stationary biosafety cabinets also each have a second, smaller alpha-beta port and a bottom dunk tank as alternative ways for passing materials and supplies into the cabinets and for removing waste and supplies that require sterilization.

Wilhelmsen explains that prior to the renovation, aerosol-exposed animals were removed from biosafety cabinets through a double door pass box or autoclave while contained within biohazard bags or within rodent cages draped with disinfectant-moistened cloths. The con-
tained animals were then hand-carried or transported on a cart to the animal holding room, and passed through an open room door to a gowned technician wearing respiratory protective equipment.

**Optimize Productivity**

“To maximize the new lab’s productivity, our goal is to increase aerosol exposure throughput and the number of different infectious agents and/or biological toxins that can be studied simultaneously,” says Wilhelmsen. “To do this we used an open lab design to improve traffic flow and worker efficiency.”

She adds that the layout includes two sets of hands-free operated double doors to encourage an orderly, directional traffic flow of people, materials, and animals.

Wilhelmsen also points to several ergonomic design elements within the stationary BSL-3 cabinets aimed at increasing worker efficiency by reducing fatigue. These features include angled viewing windows and slanted oval glove ports on both long sides of the cabinet.

In addition to the BSL-3 cabinets, the lab contains two open threesided aerosol equipment preparation alcoves for setting up the aerosol generation equipment and four BSL-3 cell culture rooms each with its own Class II biosafety cabinet, incubator, tabletop centrifuge, and bench space.

Doorways to the cell culture rooms use transparent, powered sliding doors to minimize the footprint of the doors compared to conventional swinging doors. The sliding doors also enable hands-free operation since they can be opened by an elbow-operated pressure plate and automatically close with infrared sensors.

**Stabilize Temperature and Sterilizing Procedures**

“Originally our design included double door autoclaves to be included as part of the Class III biosafety cabinets,” says Wilhelmsen. “However in the redesign we eliminated those autoclaves to create a more comfortable environment for animals inside the cabinets.”

USAMRIID installed instead a custom-designed bulk autoclave in the hallway just outside of the ABSL-3 lab. The chamber of the autoclave is over 7 feet high, 7 feet long, and 4 feet wide, which can accommodate a 2-over-2 nonhuman primate cage rack or comparably sized rabbit cage.

In addition to reducing the overall heat load in the suite, Wilhelmsen adds that the bulk autoclave has helped to control the amount of temperature fluctuations that must be corrected by the automated bioaerosol system. To further stabilize temperatures, USAMRIID installed exhaust ventilation hoods above the bulk autoclave and above the lab’s ultra-low freezers.

To sterilize laboratory equipment that cannot withstand the extreme heat of the autoclave, USAMRIID also has an airlock decontamination chamber that can be used for fumigating equipment and supplies and as an emergency backup to the autoclave. The airlock is equipped with air pressure resistant doors having inflatable gaskets, so the airlock can be made to be gas tight.

**Maximize Flexibility**

“Currently this aerobiology lab is registered to work with select agents and toxins within BSL-3 containment,” says Wilhelmsen. “However we tried to design the facility to easily accommodate future shifts in our research mission or changes in protocol or procedure.”

The stationary biosafety cabinets are considered by USAMRIID to be flexible design elements since the cabinets can be easily relocated if necessary to a new facility. In addition, the rapid access ports on the cabinets can be resized, and decontamination ports are included so that vaporized hydrogen peroxide or vaporized formaldehyde or other sterilants can be used if available in the future.

USAMRIID also provided for capped-off steam piping above the dropped ceiling over the biosafety cabinets to allow for optional installation of integral double-door autoclaves on the cabinets if steam sterilization of waste is required in the future. Quick-clamp connections were also installed to make it easy to connect the biosafety cabinets to piped utilities if necessary.

**Safety Lessons Learned**

Safety elements within the new suite include an emergency shower and eyewash plumbed in with a mixing valve to provide tempered water, an emergency phone, and a fire extinguisher. There is also an alarm that signals when pressure in the carbon dioxide gas system is low. The alarm is connected to a bank of CO₂ cylinders located outside of containment to supply the incubators and euthanasia chambers for the animals.

“A key safety challenge we face is maintaining a negative airflow gradient within this facility without getting any positive pressurization,” says Wilhelmsen. “It was difficult during the commissioning process to balance the laboratory variable flow damper system in this new lab with the building constant flow damper system in the rest of this containment building which consists of three floors of BSL-3 containment.”

Proper air balancing was achieved, however, and the laboratory has not experienced any incident of sustained positive pressurization since the start of aerosol operations in the new facility.

Wilhelmsen also points to the installation of dump valves within the airlock contamination chamber in order to deflate the air pressure resistant doors so that the air-
lock can be used as emergency egress. Otherwise the air-lock would not open, trapping anyone who was inside.

"Another critical safety feature is the establishment of a definitive one-way traffic flow of people, animals, and materials within the lab," says Wilhelmsen. "Originally we had two sets of swinging doors, but we quickly learned it was necessary to designate one set for entry only and the other set for exit only in order to have an efficient flow."

She also cites the last-minute addition of a hands-free handwash sink near the lab’s main exit as a safety element that was originally overlooked. According to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 4th edition, the sink was necessary to receive approval for handling select agents and toxins.

**Biography**

Catherine Wilhelmsen received her doctor of veterinary medicine (DVM) from Cornell University in 1978, and a PhD in veterinary pathology from Iowa State in 1989. She is a Diplomate of the American College of Veterinary Pathologists. Dr. Wilhelmsen is a 20-year veteran of the U.S. Army Veterinary Corps and served at the Armed Forces Institute of Pathology, Walter Reed Army Institute of Research, and the 10th Medical Laboratory in Landstuhl, Germany, USAMRIID, and the Armed Forces Radiobiology Research Institute. Since retiring from active military duty, she has worked as a civilian employee at USAMRIID, with successive appointments as microbiologist, biological surety officer, and microbiologist-biological safety officer. She is a certified biological safety professional (CBSP), and is licensed to practice veterinary medicine in the state of Maryland. She has consulted to the U.S. Department of Agriculture and CDC on biosafety and biosecurity, and does research and publishes on biological toxins and containment issues.

This article is based on a presentation given by Catherine Wilhelmsen at Tradeline’s International Conference on Biocontainment Facilities in March 2006.

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Autoclave: Getinge, USA Inc., Rochester, New York
Civil and Structural Engineer: NuTec Design Associates, York, Pennsylvania
Class II BSC’s: The Baker Company, Sanford, Maine
Class III BSC’s: Germfree Laboratories Inc., Ormond Beach, Florida
Commissioning Agent: USAMRIID Facilities Management/Safety
Engineering: NuTec Design Associates, York, Pennsylvania
Fire Protection Engineer: NuTec Design Associates, York, Pennsylvania
General Contractor: John J. Kirlin, Special Projects Division, Rockville, Maryland
Ultra Low Temperature Freezers: ThermoElectron doing business as Revco, Ashville, North Carolina
Three stationary free-standing BSL-3 biosafety cabinets are included in the new lab so that three different infectious agents and/or toxins may be aerosolized at one time. (Photo courtesy of USAMRIID, Photographer Larry Ostby.)

Unique mobile BSL-3 biosafety animal transporters connect to the stationary biosafety cabinets through rapid access ports. (Photo courtesy of USAMRIID, Photographer Larry Ostby.)

Dr. Wilhelmsen reads a printout of the data cycles in USAMRIID’s custom-designed bulk autoclave outside of the ABSL-3 lab. The chamber of the autoclave is over 7 feet high, 7 feet long, and 4 feet wide, which can accommodate a 2-over-2 nonhuman primate cage rack or comparably sized rabbit cage. (Photo courtesy of USAMRIID, Photographer Larry Ostby.)
Biosafety Tips

Karen B. Byers

Dana Farber Cancer Institute, Boston, Massachusetts

Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

Outbreaks of Zoonotic Infections in Rodent Facilities: Part III

The history of infections associated with rodent colonies provides invaluable insights into the development of current standards for animal facilities and husbandry practices. Published reports on LCMV infections in immunodeficient mice were summarized in Volume 10, Number 4 of this journal. In Volume 11, Number 1, the column described an outbreak of hemorrhagic fever with renal syndrome associated with Hantavirus infection that resulted from aerosolization during carcass maceration. This column augments these earlier reports of zoonotic outbreaks in rodent facilities with a summary of 126 cases of occupationally-acquired hemorrhagic fever with renal syndrome (HFRS) associated with Hantavirus infection. These cases were identified in a retrospective survey of 33 animal facilities in Japan; infections occurred at 20 medical schools, one veterinary school, and one pharmaceutical company (Kawamata, et al., 1987). All infected staff members were animal caretakers or researchers who had handled rodents. Surveys were begun in the 1970s and the association between HFRS and laboratory rodents was published in 1978.

Parenteral Exposures

Only two parenteral exposures were recorded. One needle stick occurred during blood collection and the other was a rat bite through a researcher’s glove during a weighing procedure.

Aerosol Transmission

The survey revealed that, with the exception of the two cases of parenteral exposure mentioned above, once an animal colony became infected with HFRS virus, there was a risk of airborne transmission of the virus using the practices in place at that time. The specifics are illustrative.

• A fatal infection in an animal caretaker occurred in a medical college where hanging cages were used to house rodents, and the paper sheets under the cages were changed without taking any precautions, in a manner described as “careless.” The authors assumed high levels of contaminated airborne dust, combined with the low humidity in the room, resulted in inhalation of the pathogen. After the fatality, blood was drawn from 34 of the 1,200 rodents in the room and 28 were seropositive for HFRS. Two other animal caretakers who worked in the same room had subclinical infections.

• Two animal caretakers became infected when they defrosted a freezer used to store rat carcasses. Some of the bags containing carcasses had leaked and it was assumed the cleaning operations aerosolized the virus.

• Further analysis of the 126 laboratory-acquired HFRS infections in this survey revealed that 108 were laboratory researchers performing experiments with rats and 18 were animal caretakers. The authors speculated that the animal caretakers may have had mild subclinical infections and became immune. One example cited was a case of HFRS diagnosed in a physician who entered a contaminated animal room for 10 minutes, but did not handle the animals.

• In one medical school, the rodent serological survey revealed HFRS infection was limited to two rooms where commercially-obtained rats were housed. Breeding of other rats occurred in different rooms of the same facility. Dirty cages from all the rooms were co-mingled in the “dirty” corridor on their way to the facilities’ one wash area, where cages were cleaned, but not sterilized. There was no attempt to segregate the cages from the various rooms. Despite the potential for environmental transmission from these cages, the rodent infections remained localized to the two rooms dedicated to housing the commercially-obtained rats.
Conclusions Made on HFRS Virus Transmission and Control

It is not known how the Japanese rat colonies became infected; however, wild rats were observed in an animal facility where infections occurred. This was considered a probable source since the virus causing HFRS was present in the wild house rat population, and had caused a community outbreak of HFRS in Osaka in the 1970s. In addition to exposure to wild rodents, rodent colonies were potentially contaminated by 1) introduction of infected commercially-obtained rodents, 2) sharing of animals between institutions, and 3) injection of contaminated tumors or cells into rodents. The infectious agent for this disease was first isolated in 1978 and an IFA identification method was published in the literature; making screening rodent colonies easily feasible.

Today, accepted practices for rodent colony maintenance include serological screening, micro-isolator cages or ventilated cage racks, stringent ventilation and humidity control, and husbandry practices that minimize aerosolization. However, we should all remember that rodent colonies present unique occupational environments and the scientific community must always carefully assess the potential for microbial transmission within their colonies.

References


Ask the Experts

John H. Keene
Biohaztec Associates, Midlothian, Virginia

Do you have a biosafety question and you’re not sure who to ask? Send your questions to the “Ask the Experts” column and I’ll get them answered for you. Drawing from my own experience or that of other experts in the field, we’ll try to compile a thorough and comprehensive answer to your question. Please e-mail your questions to jkeene@biohaztec.com or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Disinfectants, What Kind and When?

Questions have arisen about the potential for development of resistance to antibiotics and disinfectants in the laboratory along with the use of these agents to clean up spills and decontaminate laboratory surfaces. In addition, there have been concerns about the efficacy of antimicrobial soaps for hand washing. In this article, we will attempt to clear up some of the questions regarding the use and abuse of disinfectants.

Can microorganisms develop resistance to disinfectants, as they seem to do against antibiotics?

The mechanism of action of disinfectants and antibiotics is significantly different. Disinfectants are hazardous chemicals that destroy things like proteins, lipids, nucleic acids in living organisms (all living organisms—you, me, experimental animals, bacteria, etc.). Destruction of one’s proteins by hazardous chemicals is difficult to overcome. Therefore, while there may be some increased tolerance to disinfectants by organisms, the concentrations used generally exceed the lethal dose of the agent and the disinfectant still works.

Antibiotics generally work by interfering in the metabolic pathways of those organisms that are susceptible. Interference with metabolic pathways can be overcome when there are mutant organisms already present in the population and these are actually selected for by the presence of the antibiotic in the environment, at which point the antibiotic is no longer effective (Rice, 2004). Given the mechanism of action of each type of agent, it is much more likely that resistance to antibiotics will develop than true resistance to disinfectants.

What type of disinfectant should be used in the laboratory?

There are two answers to this question, in spite of the fact that the OSHA Bloodborne Pathogen Standard states that an EPA registered disinfectant with Tuberculocidal activity is required. (For a complete list of the EPA registered disinfectants, see www.epa.gov/oppad001/
There are a number of other parameters that must be taken into account. A full review of the selection and use of disinfectants, as well as modes of action of disinfectants, can be found in Hospital Epidemiology and Infection Control (Rutala & Weber, 2004) and Disinfection, Sterilization and Preservation (Block, 2001).

How effective are “antimicrobial soaps” for hand washing?

For the most part, the use of disinfectant soaps is a “sales gimmick” since, as mentioned above, the antimicrobial activity of any disinfectant requires an exposure time, which is generally longer than most of us wash our hands. The purpose of hand washing is to physically remove the dirt, grime and microorganisms from your hands. The “detergent” present in the hand soap is probably the most active ingredient since that is what loosens up the materials and allows the water to wash it away. The disinfectant action takes place in the sewer line, not on your hands. THE SOLUTION TO POLLUTION IS DILUTION.

With regard to the use of common hand disinfectant techniques, three things should be remembered:

1. Alcohol wipes will be active against most surface skin bacteria, but only at the right concentration, and are not active against all bacteria and viruses. (There is no such thing as a universal disinfectant that is completely safe for use on your skin.)

2. Hand cleaning foams that contain alcohol or other disinfectant also contain some type of detergent-based material for loosening the dirt and grime. The disinfectant works in the trash can or sink trap, not necessarily on your hands.

3. The Quaternary ammonium (Quats) disinfectant wipes are not always effective since we know that many organisms will actually grow in these disinfectants. In addition, Quats are surface acting agents and their antimicrobial activity in hand washing is most likely related to the fact that they have a detergent action and the “germs” are physically removed, than to their actual affect on the organisms on the hands.

Many eons ago, in another life as a clinical microbiologist and infection control practitioner, we actually stopped an epidemic of hand-transmitted urinary tract infections in an ICU by removing the “antimicrobial” soap the nurses were supposed to use, but didn’t because of the harshness of the agent. We replaced it with a mild hand soap which was easy on the hands and smelled nice. It didn’t kill anything, but the staff used it, and physically removed the offending organisms.

Summary

Unlike the development of resistance to antibiotics by microorganisms, these organisms are unlikely to de-
velop sufficient tolerance to disinfectants to result in failure of the efficacy of the disinfectant. A number of factors must be considered when choosing a specific disinfectant for use in the laboratory. Antimicrobial soaps actually have limited usefulness.

References


Corrections and Clarifications

Table 4 was omitted in the article “Some Bioterrorism Issues of Quantitative Biosafety” by Alexander Sabelnikov et al., in Volume 11, Number 2, 2006. Information in Table 4 is as follows:

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The article “Vaporized Hydrogen Peroxide Based Biodecontamination of a High Containment Laboratory Under Negative Pressure” by Jay Krishnan et al. in Volume 11, Number 2, 2006 has incorrect paragraph headings on page 75. The first heading should read “Preparation for Biodecontamination” and the second heading should read “Biodecontamination Program Cycle.”
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Marriott Copley Hotel, Boston, Massachusetts
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

November 13-14, 2006
San Antonio, Texas
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October 18-21, 2009
American Biological Safety Association (ABSA) 52nd Annual Conference
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EPA Pesticide Program Updates from EPA’s Office of Pesticide Programs—3/28/06

New Telephone Line Established for Information about Antimicrobial Pesticides

The National Pesticide Information Center (NPIC) is now taking inquiries, via their telephone help-line and web-based services, regarding antimicrobial pesticides and pesticide products. Therefore, EPA’s Antimicrobial Division hotline has been terminated, and EPA web pages with references to the Agency hotline are being updated to reflect this change.

NPIC is a toll-free telephone service that provides objective, science-based information about a wide variety of pesticide-related subjects. The service is available daily, 6:30 a.m. - 4:30 p.m. (PT), and the toll-free phone number is 1-800-858-7378.
For more than 20 years, since it was first published in 1983, the Laboratory Biosafety Manual has provided practical guidance on biosafety techniques for use in laboratories at all levels.

For this new edition, the manual has been extensively revised and expanded. The manual now covers risk assessment and safe use of recombinant DNA technology, and provides guidelines for the commissioning and certification of laboratories. Laboratory biosecurity concepts are introduced, and the latest regulations for the transport of infectious substances are reflected. Material on safety in healthcare laboratories, previously published elsewhere by WHO, has also been incorporated.

Electronic versions available from a link on the absa web site “resources”, then “biosafety guidelines” or at www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Available In
English - [pdf 1.34Mb]
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—Part II-Biosecurity Guidelines
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—Part VI-Chemical, fire, and electrical safety
—Part VII-Safety organization and training
—Part VIII-Safety checklist
—Part IX-References, annexes, and index

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www.biosafety.anl.gov/IBC_BiosafetyLinks.htm

A thorough collection of references and incident case studies for the biosafety program are available at this site. Topics covered are:

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**Viewpoints**—Short articles focusing on personal experiences may be submitted to this section. Articles vary in length.

**Book and Video Reviews**—Reviews of books and media (videos/CD/other) of interest to biological safety or biosecurity may be submitted at any time. Reviews typically do not exceed 1-2 pages in length. Books or media which authors wish to have considered for review may be sent directly to the ABSA National Office.

**Commentary/Editorial**—Brief comments on submissions published in *Applied Biosafety*, issues critical to the profession and practice of biological safety, or letters to the Editor may be submitted to this section. Individuals may be invited by the Editors to submit a guest editorial article.

**Presentations**—Articles that recount or summarize information relevant to the field of biological or biosecurity that has been presented at a conference. Presentation articles vary in length.

Other Requirements

1. **Send an electronic submission or one (1) typeset copy with a disk** of each submission to: **Editor, Applied Biosafety: Journal of the American Biological Safety Association, c/o ABSA, 1200 Allanson Road, Mundelein, IL 60060-3808, USA.** Submissions that are under consideration by another periodical or publisher or submissions that have been previously published must be identified as such, and previous citing must be disclosed.

2. **Submission guidance:**
   - Format for 8-1/2” x 11” paper using 1” margins, double-spacing, and full-justification.
   - References, footnotes, table captions, and quotations should be single-spaced, a guide to references can be found at www.absa.org.
   - Use Times New Roman, Arial, AvantGarde, Helvetica, or Universal font in 12 point.
   - Use high resolution laser printing if submission is made in hard copy media.
   - Primary headings should be flush left, bolded, and have the first letter of all main words capitalized throughout the submission.
   - Secondary headings should be flush left, italicized, and have the first letter of all main words capitalized.

3. **Submissions should follow ASM guidelines regarding fundamental style and ethics**—refer to *Applied and Environmental Microbiology* at http://aem.asm.org.

4. **The Attention Authors Form** may serve as a cover sheet with the full name(s) and degree(s) of the author(s), professional affiliations, and the return mailing address of the author to whom correspondence can be sent. Authors’ names, positions, titles, and places of employment should not appear in the body of the paper to facilitate the blind review process.

5. **Tables, charts, photographs (at least 3-1/2” x 5”) or diagrams must be computer-generated or professional quality and submitted as camera ready artwork.** Tables, charts, or diagrams should be submitted on a separate page, referenced back to the text in a vertical (portrait) format including any legend, label, or number associated with them. Refer to each as Table 1, Table 2, etc., centered above the table. Captions should be single-spaced.

6. **It is the author’s responsibility to secure written permission from the original copyright holder to use quotations of over 300 words from one source or use adaptation of tables or figures from copyrighted sources.** A copy of the copyright holder’s written permission must be provided to the Editor immediately upon acceptance of the submission for publication. The author(s) bear full responsibility for the accuracy of all results, references, quotations, and materials accompanying their submissions.

7. **In the event a diskette it used, it should be prepared on either an IBM or IBM-compatible computer.** All submissions should be formatted using either: Microsoft Word, Microsoft Publisher, or WordPerfect. ASC II files are also acceptable.
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Checklist

☐ Electronic submission or one (1) hard copy (typeset using 8-1/2” x 11” paper with 1” margins).

☐ Electronic submission or one (1) hard copy original of tables, figures, and/or illustrations.

☐ Submission follows ASM guidelines regarding fundamental style and ethics.

☐ Abstract of approximately 250 words (for Articles, Reviews, and Summary Articles only).

☐ This Attention Authors form.

Author’s Signature_________________________ Date_________________________

Please send the completed form along with your submission to: Editor, Applied Biosafety: Journal of the American Biological Safety Association, 1200 Allanson Road, Mundelein, IL 60060-3808, USA. Electronic submissions may be e-mailed to: Production Editor, Karen D. Savage, at ksavage@covad.net. If you have formatting, processing, or general questions, please contact Ms. Savage at the ABSA National Office Monday through Friday between 9:00 a.m. and 5:00 p.m. Central Time at 847-949-1517.
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