Risk Assessment of Biological Hazards

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RISK ASSESSMENT OF BIOLOGICAL HAZARDS

Biosafety is an inexact science, and the interacting systems of agents, activities, and the people performing the activities are constantly changing. Work with biohazardous agents, or materials suspected of containing such agents, needs to be assessed for the risk it poses to the worker, the community, and the environment. Whether the work is to be done in research, diagnostic, or teaching laboratories or at large scale for production in industrial facilities, a risk assessment should be done to provide the information needed to eliminate a particular risk or reduce that risk to an acceptable level. The assessment of risk needs to be carried out by a knowledgeable person using professional judgment and common sense. Using valid information about the specific agent and taking into account any additional risks posed by the specific procedures and equipment, the assessor should be able to identify the most appropriate work practices, personal protective equipment, and facilities to protect healthy adult workers and the environment. A risk assessment should be done before work begins and should be repeated when changes are to be made in agents, practices, employees, or facilities. The risk assessment for work with biohazardous agents must take into account not only the agent but also the host (worker) and the environment (activity/community).

This chapter focuses on agent- and activity-based risk assessments. Host factors are addressed briefly, but they are more appropriately covered under occupational medicine (chapter 24). The risk/threat assessment required for handling select agents (Department of Health and Human Services [DHHS], 2005; Department of Agriculture [USDA], 2005) is addressed separately, in chapter 33. Information on the risks associated with many biohazardous agents and materials can be found in the chapters to follow; information on practices, equipment and facility design for biohazard control are addressed in section III of this book; further information on host factors is provided in chapter 24.

THE ASSESSOR

Assessing the risks associated with biohazardous agents, or obtaining such an assessment, is the responsibility of the supervisor or his or her designee, as determined by the institution. An appropriately trained professional (microbiologist, sanitarian, industrial hygienist, infection control practitioner, veterinarian, etc.) is needed to assess the risk associated with the agent. The American Biological Safety Association (Mundelein, Ill.) maintains a list of certified biological safety professionals who may

be of assistance to those who lack such expertise. Staff from the Centers for Disease Control and Prevention (CDC), in the Bacterial and Mycotic Diseases Branch of the Center for Infectious Diseases (CID), are also willing to provide information on human pathogenicity of known strains of microbial agents (personal communication, CDC, CID). The risk associated with protocols, activities, and equipment is best addressed by the workers most familiar with the equipment and procedures to be used (World Health Organization [WHO], 2004, chapter 2). This is especially true as new technologies generate unexpected problems which can lead to unanticipated new risks. The control of biohazardous agents based upon risk should be made flexible by giving permission to the knowledgeable user to vary the containment according to the specific virulence factors of the organism being used, and the assessment of the hazards associated with the tasks.

RISK ASSESSMENT: THE BIOHAZARDOUS AGENT

Assessing the risk of work with biohazardous agents is not as straightforward as that for inanimate chemical and physical hazards. Biohazardous microbial agents exist in a variety of environmental niches and can express different virulence factors in dynamic host-parasite interactions. For some agents of human disease, such as hepatitis B virus (HBV), we do not even know the nutritional or host cell requirements that would allow their propagation. Although biohazardous agents do not fit into rigid categories, it is possible to assess the relative risk of an infectious microorganism and to place the wild type of that genus or species into one of four risk groups (RGs). Variants, types, or strains of that species need to be assessed for the risk associated with the variation from the wild type. The RG is the classification of an organism based on known factors. It can be used to begin a risk assessment based on what is known about the agent.

RGs

The WHO provided the basic definitions for the classification of infective microorganisms by RG in their *Laboratory Biosafety Manual,* first published in 1983. The second and third editions of the manual continue to encourage countries or regions to classify agents which could be encountered there (WHO, 2004), taking into account factors such as pathogenicity, modes of transmission, and host range of the organism, as influenced by existing levels of immunity in the local population and the density and movement of the host population; the presence of appropriate vectors; standards of environmental hygiene; and the local availability of effective preventive measures. According to the WHO, these preventive measures could include sanitary precautions, e.g., food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; the importation of potentially infected animals or animal products; and prophylaxis by vaccination or antiserum. This could include passive immunization and postexposure vaccination, as well as the use of antibiotics, antivirals, and chemotherapeutic agents, taking into consideration the possible emergence of resistant strains (WHO, 2004). The many factors to be considered in compiling a list of agents mean that the lists will vary from country to country and should only be applied in the country of origin.

In the 2004 revision of the manual the WHO, aware of the tendency to misuse these lists, cautioned that simple reference to the risk grouping is not sufficient for a risk assessment; other factors that need to be considered include not only the pathogenicity of the specific strain of the agent and the infectious dose but also the potential outcome from an exposure; the natural route of infection and other routes which could result from laboratory manipulations; the stability of the agent in the environment (inherent biological decay rate) (WHO, 2004); the concentration of the agent and the volume to be manipulated; the presence of a suitable host (human or animal); information from animal studies or reports of laboratory-acquired infections, as described in chapter 4 of this book; laboratory activity planned (homogenization, sonication, centrifugation, aerosolization, etc.); any genetic manipulation which could extend the host range or alter sensitivity to known effective treatment; and local availability of appropriate treatment or prophylaxis.

The prevailing conditions in the geographical area in which the microorganisms are handled must also be taken into account. Individual governments may decide to prohibit the handling or importation of certain pathogens except for diagnostic purposes. Competent authorities in Australia, Canada, and the European Union (EU) and the National Institutes of Health (NIH) guidelines in the United States have placed biohazardous agents into four defined groups reflecting an increasing risk to the user and the environment. The WHO manual provides general guidance on biosafety, taking into account factors that affect its international application, specifically, the difference in the risks attributed to certain biological agents and the availability of appropriate laboratory facilities and trained staff (WHO, 2004).

In the RG definitions provided in the appendix of this chapter, the WHO, Australia, and Canada have included not only the risk to humans but also the risk to livestock and the environment due to economic concerns. Australia also added the risk to plants of economic importance. In assessing risk, Canada took into consideration the economic impact on the environment, including plants, but did not include plant pathogens in their list of pathogens according to RG (Laboratory Centre for Disease Control, Health Protection Branch, Health Canada, 1996). The latest publication from Canada (Office of Laboratory Security, Health Canada [OLS], 2004) does not include a printed list of human and animal pathogens. An updated list is to be made available from the OLS on its website http://www.hc-sc.gc.ca/pphb-dgspsp/ols-bsl/. The concept of RGs remains; however, in Canada the static list has been replaced by a mechanism for access to a current list. The EU and the United States limit their lists of pathogens to those causing disease in healthy adult humans, the workers at risk. Since the definitions are not identical for all countries, the lists will differ accordingly.

Although in the United States, *Biosafety in Microbiological and Biomedical Laboratories* (also known as BMBL) does not actually use RGs, the description of agents to be handled at each of four biosafety containment levels (BSLs) includes characteristics which allow them to be interpreted as RG definitions (CDC/NIH, 1999). The protection of animals, plants, and the environment is not mentioned at BSL-1 and -2, where the focus is on the protection of workers. Community risk is addressed at BSL-3, with aerosol-transmitted agents to be handled under primary containment which, when combined with the secondary containment provided by the facility, is meant to reduce or eliminate release to the surrounding community. BMBL describes four BSLs to provide actual containment criteria for agents known to have caused laboratory-acquired infections, those which could reasonably be expected to do so, and those which would have serious consequences.

The NIH guidelines for research with recombinant DNA molecules, a separate guideline in the United States, do provide definitions for each of four RGs (NIH, 2002). This guideline, as well as those from Australia, Canada, and the EU, does not mention aerosols in the definition of an RG 3 agent. This important variation results in the inclusion of human immunodeficiency virus (HIV) in RG 3 in the lists provided by those countries, while the containment conditions recommended in BMBL for the use of HIV, or clinical materials suspected of containing HIV, range from BSL-2 to BSL-3. This subtle difference in risk management causes great consternation among those who would put infectious agents into rigid classification schemes. These variations cause concern because the list has been taken at face value without reading the accompanying document (Commission of the European Communities, 2000). In the EU directive, introductory note 8 in annex III states, "Certain biological agents classified in group 3 which are indicated in the appended list by two asterisks (**), may present a limited risk of infection for workers because they are not normally infectious by the airborne route. Member States shall assess the containment measures to be applied to such agents, taking account of the nature of specific activities in question and of the quantity of the agent involved, with a view to determining whether, in particular circumstances, some of these measures may be dispensed with." Looking at the list of viral agents in RG 3 in that document, one finds HIV, human T-cell leukemia virus types 1 and 2, HBV, HCV, and rabies virus, among others, in this group. The United States, in BMBL, handled the situation more directly. Indeed, although BMBL appears to be an outlier in not defining RGs, it does provide flexibility in risk management via the BSLs recommended in the agent summary statements. For example, since HIV has not been shown to be transmitted by the aerosol route, BSL-2 containment is appropriate for clinical labs. Amplification of HIV by cocultivation increases the risk of exposure and leads to an increase in the level of containment to include BSL-3 practices with no requirement for a BSL-3 facility. The EU and BMBL merely reflect two approaches to risk assessment which can reach the same conclusions for risk management. HIV is, by the WHO, Australian, Canadian, and EU definition, in RG 3, but they agree with BMBL that it can be safely handled at either BSL-2 or BSL-3, according to the activity. This situation highlights the importance of doing not only an agent-based risk assessment but also, more importantly, a protocol-driven risk assessment. It also shows that the RG of an agent can be related to but does not have to equate with the biosafety containment level (WHO, 2004, Table 2). Although this difference in listings of agents according to RG is to be expected when following the WHO guidelines, this lack of global uniformity has not been well received in the United States. The disparity must be interpreted appropriately by those who assess the risk of work with biohazardous agents at academic, government, and industrial sites in different countries. Those who do not understand the EU directive assess the risk by using only the RG listed for the agent, thus erroneously assuming that BSL-3 practices and facilities are required for all work with HIV in the EU.

In assigning an agent to an RG, one must also take into account that "there are in all groups of microorganisms naturally occurring strains which vary in virulence, and may thus need to be handled at a higher or lower level of containment" (Commission of the European Communities, 2000). Exotic, restricted agents differ from one country to another; thus, the lists of those agents placed in the four RGs differ among countries. Agents which have not been assessed for risk or which have not been listed by the competent authority of a participating country should not automatically be considered nonhazardous. A default risk assessment of a minimum of RG 2 and the use of standard microbiological precautions can be found in the Australian, Canadian, and EU guidelines mentioned above. In the United States, this would be the use of "universal/standard precautions" for all human secretions and excretions, which translates as BSL-2 in the laboratory (OSHA, 1991; CDC/NIH, 1999, p. 157–158).

Many of the agent factors to be considered in a risk assessment were mentioned above, some of which formed the basis of the definitions for RGs (see the Appendix), while others are to be considered in the overall risk assessment. An initial agent-based risk estimate for work with a microorganism can be predicated upon information provided in guidelines available from government agencies, professional associations, academic institutions, or designated competent authorities (Brooks et al., 2004; CDC/NIH, 1999; Commission of the European Communities, 2000; Fleming and Hunt, 2000; Heymann, 2004; Kuenzi et al., 1985; NIH, 2002; OLS, 2004; Standards Australia/Standards New Zealand, 2002; WHO, 2004). Those countries providing a WHO RG classification have published their lists of pathogens, or will make them available on the Internet, ranked according to their definition of RGs 1 to 4 (see the appendix). The list for the EU is included in a directive issued for the protection of workers from exposure to biohazardous agents (Commission of European Communities, 2000).

The agent summary statements in BMBL provide the risk assessment and containment recommendations for the use of agents which have been reported to cause laboratory-acquired infections or could be expected to have a serious outcome (CDC/NIH, 1999). Many potentially biohazardous agents are not included. For example, *Bacteroides fragilis*, *Enterobacter aerogenes*, *Haemophilus influenzae* type b, and *Staphylococcus aureus* are not covered by agent summary statements because they have not been shown to pose a serious hazard to a healthy adult as a laboratory-acquired infection. However, in doing any risk assessment, care must be taken to determine the true disease potential of an agent in the activity proposed. Information on human communicable diseases is provided in the *Control of Communicable Diseases Manual* from the American Public Health Assocation (Heymann, 2004), and the relative risk of isolates from human clinical samples is also available in the literature (Isenberg and D'Amato, 1995; Brooks et al., 2004). Lists of biohazardous agents which require special practices in handling, packaging, and transporting are provided by the CDC (42CFR Section 72), as the government agency responsible for identifying biohazardous agents which are to be regulated in transport as well as the packaging needed for the import and transport of such agents (see chapter 21, on shipping). The DHHS and the USDA have provided lists of agents and toxins which are restricted under new regulations to prevent their use in bioterrorist activities (DHHS, 2005; USDA, 2005). See chapter 33.

Information collected in the risk assessment may confirm an increased virulence of the specific strain or serotype in use, in which case the risk assessment may be altered enough to require an increase in the level of containment. For example, organisms which have developed resistance to multiple therapeutic drugs, such as *Mycobacterium tuberculosis* (MDRTB), are considered to be of a higher risk due to the lack of treatment alternatives and are to be handled with more stringent precautions. This organism is in RG 3, but the extra precautions required for safe work with MDRTB would not be expected to take it to a higher containment level than BSL-3. Conversely, if the assessment indicates a lower level of virulence, a relaxation of some of the protective measures may be in order. For example, an avirulent strain of the same microbe, *M. tuberculosis* H37Ra, has been handled safely under BSL-2 containment, even though the virulent strain H37Rv is to be handled at BSL-3. *Streptococcus pneumoniae* is in RG 2. Strains exist which no longer possess the capsule, a virulence factor which allows this pathogen to evade the phagocytic arm of the host immune system. These strains, which are nonpathogenic because they are readily cleared by host defenses, can be handled safely at BSL-1. The need for a knowledgeable assessor cannot be overemphasized.

Yersinia enterocolitica, a bacterial agent of enteric disease, is known to have specific invasion genes as virulence factors, but it is also known to have strains which are nonpathogenic (Miller and Falkow, 1988; Miller, 1992; Finlay and Falkow, 1997). A rigid agent classification system would impose a higher level of containment than is necessary for work with the nonpathogenic strains of pathogenic organisms. There should be enough flexibility in the

criteria to allow such strains to be handled and shipped as nonpathogens even though the wild-type strain belongs in an RG which requires a higher level of containment and special packaging.

Microorganisms attenuated for use as killed or live vaccines may no longer require the same containment as the wild-type, parent organism (Commission of the European Communities, 2000, directive 2000/54/EC, annex III, no. 4, L262/29). For example, some attenuated influenza virus vaccine strains may be handled at BSL-1, while work with the parent virus is done at BSL-2. Highly pathogenic avian influenza virus (HPAI) strains are considered for even higher levels of containment to protect the community from a pandemic. The strain of *Mycobacterium bovis* known as the bacillus of Calmette and Guérin (BCG) is handled at BSL-2, but the wild-type RG 3 *M. bovis* strain is more virulent and usually requires BSL-3 work practices and facilities. Similar assessments have led to exemptions from registration of vaccine strains or inactivated preparations of selected agents (CDC, 2002). Recent laboratory exposures to *Bacillus anthracis* and *Francisella tularensis* have identified a need for suppliers and recipients of microorganisms to confirm by appropriate tests that an organism that is supposed to be attenuated or killed actually is.

A rigid classification of risk would inadvertently exclude the use of higher containment for situations in which the virulence of a pathogenic agent has been reassessed or significantly enhanced. The live poliovirus vaccine strains (OPV), which were given orally to children and adults, were once considered safe to handle at BSL-1, although the wild-type strains of polio virus were handled at BSL-2. When vaccine-associated cases of polio began to occur more frequently than naturally occurring cases, the oral live polio viral vaccine (OPV) began to be replaced by the inactivated polio vaccine (IPV) on the recommendation of the Advisory Committee on Immunization Practices (CDC, 1999, 2002, 2003, 2004). The WHO recommended a program in which stocks of poliovirus were to be destroyed in the future in anticipation of global eradication of the virus by 2005. Due to recent outbreaks, that timetable has been revised. At present, the poliovirus is to be handled at BSL-2/ polio. Once the poliovirus is eradicated in the wild, laboratories wishing to work with wild poliovirus or infectious or potentially infectious materials will do so under BSL-3/polio. The specific requirements for this level of containment are meant to be applied only to work with the poliovirus. When oral poliovirus immunization stops worldwide, the WHO plans to reclassify wild poliovirus as an RG 4 agent to be handled under BSL-4 maximum containment. The OPV virus stocks will be handled at BSL-3/polio (WHO, 1998, 1999). At that time, the only source of poliovirus will be laboratories and vaccine production facilities. The relative risk of reintroduction of poliovirus from a variety of sources in such facilities has been assessed and is provided in Table 1. The most important source of virus for reintroduction is facility personnel, who should thus be the focus for management of the risk of release to the community (C. Wolff, D. O. Fleming, and W. Dowdle, 2005, Assessment and management of post-eradication poliovirus facility associated community risks. Presented at the 48th Annual Biological Safety Conference, Vancouver, Canada).

TABLE 1 Risk of poliovirus reintroduction from laboratory or vaccine production facilities[a]

Potential viral source	Relative risk
Facility personnel	
Infected	++++
Contaminated	++++
Liquid effluent	− to +++
Air effluent	+/−
Solid-waste disposal	+/−
Materials in transit	+/−
Laboratory animals	−

[a]From Wolff et al. (48th Annu. Biol. Safety Conference, 2005).

Eradication plans and the proposed changes in containment requirements must now take into account the fact that scientists have synthesized a poliovirus. (Cello et al., 2002).

RISK ASSESSMENT OF UNKNOWNS

We continue to be challenged by emerging infectious diseases, such as severe acute respiratory syndrome-related coronavirus (SARS-CoV) and HPAI (highly pathogenic avian influenza) virus, which must be assessed for risk to prevent epidemics, even as they are first being identified. Laboratory-associated infections reported with these agents suggest either that the risks were not assessed correctly or that the workers failed to use the laboratory practices needed to control those risks. We also continue to be faced with reemerging infections with agents that develop multiple antibiotic resistance, such as MDRTB and vancomycin-resistant *S. aureus*. The risk assessment of such agents changes when there is no effective treatment. In other situations, where a sample or agent has not been well characterized, there may not be enough data to allow the risk to be assessed with any confidence. The information that is available is used to develop a rational default process for handling such

unknown agents. HPAI is highly infectious and is known from the 1918 pandemic to extend its host range from avian to human. There is no proven treatment except perhaps Tamiflu; thus, release would have very serious consequences for the worker and the community. The minimum default risk assessment would require BSL-3 containment for diagnostic work, with enhanced precautions for cultures of such HPAI strains as H5N1.

In another situation, soil samples are sent to a drug discovery facility from sites around the world, which may vary from a tropical rain forest to a pigeon-infested city park. All such samples are subject to a risk assessment because pathogenic agents ranging from exotic viruses to the spores of pathogenic fungi, such as *Histoplasma capsulatum*, may be present. It is prudent to consider such soil samples as potentially contaminated with organisms indigenous to the area, using BSL-2 at a minimum (WHO, 2004), and to process the sample so as not to expose workers to such agents by minimizing the production of and exposure to splash, spatter, and true infectious aerosols (CDC/NIH, 1999, Section VII-B).

ACTIVITY-BASED RISK ASSESSMENT

Information on the exposure potential associated with specific work practices and equipment helps to identify situations which need to be controlled before the work begins. Factors to be studied prior to the proposed work include the following: (i) potential for generation of aerosols, including splash and splatter; (ii) quantity (volume, concentration/titer, infectious dose, etc.); and (iii) work proposed (in vitro, in vivo, aerosol challenge, or environmental release).

The protocols or standard operating procedures being developed for the specific tasks and equipment involving the etiologic agents of human disease can be assessed to identify the need for special containment practices or protective equipment. We are faced with the potential for exposures associated with new equipment which can produce unexpected aerosols due to the lack of preuse sham testing. We must assess work activities, such as centrifugation, homogenization, sonication, etc. A risk assessment is especially important for new procedures accompanying technological advances in related fields, such as the creation of infectious virus from its genetic blueprint. Do chemists and molecular biologists assess and understand the risk? Are such scientists aware of the outcome of exposure (Cello et al., 2002)? We must take these concerns into account in doing a risk assessment and identify the potential for exposure to be encountered in various work environments, from molecular biology to the testing of potential new antibiotics against the latest clinical isolates.

RISK ASSOCIATED WITH RECOMBINANT ACTIVITIES

Factors involved with recombinants include properties of the genes, such as codes for virulence factors, toxins, host range, integration, replication, reversion to wild type, etc. (see WHO, 2004, chapter 18).The actual process of producing a recombinant organism is not one of the factors of concern since the National Research Council (NRC, 1987) concluded that there is no evidence of any unique hazard posed by recombinant DNA techniques. They concluded that the risks associated with recombinant DNA are the same in kind as those associated with unmodified organisms or organisms modified by other means. The NRC recommended that risk assessment of work involving environmental release of recombinants be based on the nature of the organism and the environment into which it is introduced, and not on the method by which it is produced. This recommendation for risk assessment of the product rather than the process was also accepted by the Office of Science and Technology Policy (1986) and the NRC (1987). Guidelines for assessing the risk of recombinant work have been provided by the NIH (2002), Canada (OLS, 2004), and the WHO (2004). Although all possible scenarios cannot be addressed, the potential hazards may actually be novel and uncharacterized. The WHO (2004) lists the following factors to be considered in the risk assessment: properties of the donor organism, nature of the genetic sequences that will be transferred, properties of the recipient organism, and properties of the environment. There is always the risk that a more virulent organism will evolve from the insertion of a gene not normally found in that host. For example, a more virulent mousepox virus resulted from the insertion of the gene for interleukin 4 (Jackson et al., 2001). Biosafety and recombinant viral vectors are addressed in more detail in chapter 28 of this book.

SCALE-UP AND LARGE-SCALE ACTIVITIES

Work with biohazards can be divided into research, diagnostic, or large scale, with the last usually referring to levels greater than 10 liters in the United States and Canada. Volume, however, is not the sole determinant, because the intent of the work can also determine the scale in countries such as the United Kingdom (Advisory Committee on Dangerous Pathogens, 1998; Cipriano, 2002). The workplace can

create unnatural situations which increase the risk of employee exposure to infectious, toxigenic, or allergenic agents or materials. In the research lab, work is usually limited to relatively few organisms which are known to the investigator, who can usually choose the agents of interest. The diagnostic or clinical laboratory handles unknowns in clinical samples but has an idea of the agents that can be isolated from each patient site and whether or not they are known to cause human disease (Isenberg and D'Amato, 1995). Regarding work with large volumes of agents, the agent summary statements in BMBL suggest considering an increase in the level of containment (CDC/NIH, 1999), without any mention of specific laboratory precautions that could be applicable to work done in bioreactors or fermentors. Many of the agents being used in large-scale production are not covered by agent summary statements. Each manufacturer using such agents is to assess the situation and determine the appropriate containment. Appendix K of the NIH recombinant DNA guidelines (NIH, 2002) continues to provide assistance in developing specific practices for large-scale recombinant work. Biosafety guidelines for large-scale work with nonrecombinant pathogens have also been published to provide recommendations which relate to the special equipment and facilities used in scale-up and production work (Cipriano, 2000, 2002). Further information may be found in chapters 30 and 31 of this book.

AGENT-ACTIVITY INTERACTION: JSA

A thorough evaluation of the hazard potential of the work practices, procedures, and equipment to be used for proposed tasks is called a job safety analysis (JSA). In the JSA, the supervisor or his or her designee analyzes the risk of employee exposure from each task in which a biohazard is involved. Ideally, those who develop the work procedures and those who will actually perform the work are involved in the JSA in order to recommend less risk-prone options wherever possible. The job is divided into steps which describe what is to be done, instructions are reviewed or developed for each step, and key points are provided, such as warnings of a specific hazard or a potential accident. Methods of control for each potential hazard are then developed. An example of a JSA for a sterilizer/autoclave can be found in Songer, 1995.

A JSA is to be done in advance of work with hazardous agents. Information on the best work practices and the safest equipment for handling biohazardous materials, along with other hazards associated with the tasks, can then be provided to the worker prior to carrying out the procedures. The supervisor is responsible for the safety orientation and the specific training required for the safe performance of the work. The Occupational Safety and Health Administration (OSHA) standard for worker protection from blood-borne pathogens requires that information and training be provided prior to work with blood-borne pathogens and prior to offering the employee the hepatitis B vaccine, to allow informed consent (OSHA, 1991). The requirement for advance information and training should ideally be applied to all work with biohazardous agents. Directives for the protection of workers from exposure to biohazardous agents have been issued in Europe (Commission of the European Communities, 2000). Although they are regulations abroad, they can provide adequate incentive to voluntarily protect our own workers.

EXPOSURE DETERMINATION

As a part of the risk assessment process, a definition of what constitutes an exposure to the agent should be determined in advance of an incident. This allows the identification of work activities or equipment which could pose a hazard to be assessed and addressed. The definition of an exposure also prevents unnecessary medical treatment and employee concern. Individuals who are present during an incident but are not exposed to the agent by one of the routes of infection do not require medical treatment, although such individuals may need counseling or further training on principles of infection.

BIOSAFETY MANUAL

Written documentation of the risk estimate and the actual protocols are to be placed in an infection control plan or biosafety manual. Regulatory requirements for the prevention of occupationally acquired blood-borne infectious diseases in the United States now mandate such documentation in an exposure control plan (OSHA, 1991). The regulations for handling selected agents also mandate such documentation (DHHS, 2005; USDA, 2005).

HOST FACTORS: HEALTH STATUS

A more thorough risk evaluation of the agent-host-activity triad is required to develop appropriate containment for actual work with etiologic agents. After the agent and activity are assessed for risk, the remainder of the risk assessment involving the host is not the purview of the biosafety professional. From the standpoint of the biosafety professional

who cannot perform the medical evaluation of the worker, the risk of work with biohazardous agents is actually evaluated and managed on the basis of an evaluation of the agent-work activity interaction. See chapter 24 on occupational medicine and host factors.

The guidelines in the United States (CDC/NIH, 1999) presume that the worker is an immunocompetent adult. Declaration or identification of impaired host defense factors, such as immune deficiencies and extremes of age, are the responsibility of the employee. An evaluation of fitness for duty may need to be obtained from a physician, especially if there is a change in health status which could place that employee at increased risk of infection. The risk assessment needs to be kept current and relevant to the work in progress to control any potential increase in risk. The importance of evaluating the epidemiological triad (agent-host-activity) in establishing the containment level has been emphasized by the Committee on Hazardous Biological Substances in the Laboratory, NRC (1989).

Opportunistic pathogens and normal microbial flora which are of no or low risk to healthy adult coworkers can cause disease in immunocompromised or immunosuppressed adults. Frank pathogens usually pose a greater risk of more serious disease in such individuals, and the additional risk must be addressed. A similar approach is required for pregnant females, due to risk to the intrinsically immunocompromised fetus. BMBL (CDC/NIH, 1999), based upon assessment of risk, provides strong recommendations which preclude working with certain agents; e.g., serologically negative women of childbearing age should not work with *Toxoplasma.* Warnings of increased risk to the immunocompromised are also found in some of the agent summary statements (CDC/NIH, 1999). The use of potentially harmful biological agents needs to be evaluated on a case-by-case basis to prevent discrimination under the Americans with Disabilities Act (ADA, 1991). The worker must be advised of the specific hazard and understand what can be done to offer protection through a reasonable accommodation, such as a special engineering design or personal protective equipment. The worker may even be asked to sign a document indicating that he or she has been informed of the special personal hazard.

It may be difficult for the biosafety professional to understand how to comply effectively with the seemingly mutually exclusive requirements of the ADA and BMBL. Because of this, and for reasons of confidentiality, these discussions and decisions are usually handled by the worker in consultation with the occupational health physician or personal physician. When such host factors are evaluated and a task-based risk analysis is completed, an appropriately trained professional can provide advice to reduce or prevent exposure to the biohazardous agent.

ACCEPTABILITY OF THE RISK OF WORK WITH BIOLOGICAL HAZARDS

How do we assess the acceptability of the risk of work with biological hazards? Scientists cannot measure whether something is safe or not, but they are prepared to measure risk in terms of probability. Although measuring risk is an objective exercise, judging the acceptability of that risk involves value judgments and a basic knowledge of the subject. We are more likely to misjudge something unfamiliar as unsafe. A safe activity is one in which the risks are considered to be acceptable. The acceptability of a risk is constantly changing, due to changes in social values, even though the actual level of that risk might remain the same. Safety is not an intrinsic, absolute, measurable property of things. Songer (1995) cautioned us not to attempt to anticipate all of the possible risks which might occur in a work situation, such as a biomedical laboratory. He advised the use of some general, results-oriented guidelines to assess and control potential risks. For example, reduction in needle sticks is a result which can indicate that a part of the exposure control plan for the blood-borne pathogen standard is working. An increase in needle sticks could signal a defect in the program that should be identified and addressed. Using the framework provided by regulations and guidelines, we can measure risks and make safety judgments regarding the acceptability of those risks based on observed results on a day-to-day basis. Codification of risks along with prescribed requirements can adversely affect a search for better work procedures.

RISK PRIORITIZATION

A facility with limited personnel and funds should direct attention to the risks with the greatest probability of occurrence and harm. One way to prioritize risks is to develop a matrix based on the severity of the consequences and the probability that an infection will occur under the conditions of use (Table 2). The RG of the agent, which reflects agent-based risk and the relative severity of the consequences of a laboratory-acquired infection, could be used as a surrogate for a severity assessment. The probability of an accident can be estimated from the known

TABLE 2 Risk prioritization

Probability of accident	RG 1	RG 2	RG 3	RG 4
Negligible	Very low	Low	Low	Medium
Low	Very low	Low	Medium	High
Medium	Low	Medium	High	Very high
High	Low	Medium	High	Very high

hazards associated with the protocol, including the use of aerosol-generating equipment, such as sonicators and homogenizers, as well as procedures that use sharps, such as injecting animals. The probability of an accident, based on an assessment of the specific situation, would be recorded as negligible, low, medium, or high. The risk matrix provides a priority for that risk which can be read from the table as very low through very high. This risk prioritization concept in Table 2 is demonstrated in exercise 1 of Assessment and Control of Biological Agents, University of Warwick, Coventry, in the United Kingdom (D. Veale, personal communication; available online at http://www2.warwick.ac.uk/services/safety/health_and_safety/papers/bioass.pdf).

CONCLUSION

As we assess risk and attempt to translate guidelines, regulations, and standards into work practices, we should continue to search for better control methods. Rules and regulations cannot cover every possible situation (Songer, 1995). We must continue to assess the risk of biohazardous work based upon current information and to recommend appropriate, realistic methods of containment to prevent worker exposure and contamination of the environment. In the final analysis, we must be sure that those who work with biohazardous agents are trained to a level of competence and common sense which provides for their own safety and that of the community.

APPENDIX A. RISK CLASSIFICATION CRITERIA

DEFINITIONS OF RGs 1 TO 4: WHO, AUSTRALIA, CANADA, EU, AND THE UNITED STATES (CDC/NIH AND NIH)

WHO Classification of Infective Microorganisms by RG (WHO, 2004)

RG 1 (no or low individual and community risk): a microorganism that is unlikely to cause human disease or animal disease

RG 2 (moderate individual risk, low community risk): a pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

RG 3 (high individual risk, low community risk): a pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

RG 4 (high individual and community risk): a pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Australian/New Zealand Standard AS/NZS 2243.3:2002—Safety in Laboratories Part 3: Microbiological Aspects and Containment Facilities

The following classification is based on the pathogenicity of the agent, the mode of transmission and host range of the agent, the availability of effective preventive measures, and the availability of effective treatment.

RG 1 (low individual and community risk): a microorganism that is unlikely to cause human, plant, or animal disease

RG 2 (moderate individual risk, limited community risk): a pathogen that can cause human, animal, or plant disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause infection, but effective treatment and preventive measures are available and the risk of spread is limited.

RG 3 (high individual risk, limited community risk): a pathogen that usually causes serious human or animal disease and may present a serious hazard to laboratory workers. It could present a risk if spread in the community or the environment, but there are usually effective preventive measures or treatment available.

RG 4 (high individual and community risk): a pathogen that usually produces life-threatening human or animal disease, represents a serious hazard to laboratory workers, and is readily transmissible from one individual to another. Effective treatment and preventive measures are not usually available.

Canadian Laboratory Biosafety Guidelines (OLS, 2004)

Infective organisms are categorized into four RGs, reflecting their relative hazards, based upon factors such as pathogenicity, infectious dose, mode of transmission, host range, availability of effective preventive measures, and availability of effective treatment. These classifications are only applied to research laboratories or growth in small volumes for diagnostic or experimental use.

RG 1 (low individual and community risk): any biological agent that is unlikely to cause disease in healthy workers or animals

RG 2 (moderate individual risk, low community risk): any pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community,

livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease, effective treatment and preventive measures are available, and the risk of spread is limited.

RG 3 (high individual risk, low community risk): any pathogen that usually causes serious human or animal disease, that can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes diseases treatable by antimicrobial or antiparasitic agents

RG 4 (high individual, high community risk): any pathogen that usually produces very serious human disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice versa, directly or indirectly, or by casual contact

European Economic Community Directive 2000/54/EC

Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC, adopted 18 October 2000). Biological agents shall be classified into four infection RGs, according to their level of risk of infection.

Group 1: biological agent means one that is unlikely to cause human disease

Group 2: biological agent means one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community, and there is usually effective prophylaxis or treatment available

Group 3: biological agent means one that can cause severe human disease and presents a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available

Group 4: biological agent means one that causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community. There is usually no effective prophylaxis or treatment available.

NIH Recombinant DNA guidelines

Appendix B, Table 1 (United States), as revised in January 1996 (NIH, 2002)

RG 1: agents that are not associated with disease in healthy adult humans (includes a list of animal viral etiologic agents in common use in Appendix B-V)

RG 2: agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available

RG 3: agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available (high individual risk but low community risk)

RG 4: agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

CDC/NIH Guidelines

BMBL, 4th edition, 1999, Section III, gives criteria for placing work at a biosafety containment level, translated here into RGs.

BSL-1: defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans; of minimal potential hazard to laboratory personnel and the environment

BSL-2: indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity; moderate potential hazard to personnel and the environment

BSL-3: indigenous or exotic agents with a potential for respiratory transmission and which may cause serious or potentially lethal infection (applicable to clinical, diagnostic, teaching, research, or production facilities)

BSL-4: dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy

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