

BioSide Lines

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Newsletter of the Office of Biological Safety - Environment, Health & Safety, UW-Madison

www.fpm.wisc.edu/biosafety

Potential Pathogen Exposures – Prevention and Response

Prior to beginning any work involving human pathogens, a thorough risk assessment needs to be performed. The protocol submission process forms the basis for an independent assessment. This process will help you determine the proper personal protective equipment which must be used, the level of containment that is acceptable, and the specific practices that should be incorporated into your standard operating procedures. During this planning stage you should critically review all your proposed procedures and consider alternate practices that minimize the risk of exposure. Thorough planning at this stage can help prevent serious problems in the future.

In spite of our best efforts, accidents or unanticipated events can – and do – occur. If an exposure incident occurs you may need to act quickly. Planning and training are key elements in helping to deal with these incidents. You need to have an up-to-date exposure response plan. Your plan should include information on the pathogen, including symptoms and health risks associated with the agent, and specific procedures that will be utilized in the event of an exposure. It is important that all individuals receive periodic training on these procedures so that the proper actions will be taken in the event of any emergency. As part of your preparedness planning, locations of eyewash stations and safety showers should be known by everyone working in the lab, and fully stocked first aid kits must be available.

After any potential exposure, seek immediate medical attention from either the University Health Services or the UW Hospital emergency room. Your exposure response plan should specify where you should go. And don't go alone! Someone who is knowledgeable about the events and the agents involved should accompany you so that they can assist with answering questions. When talking to a medical professional, you must be able to describe the events clearly and without too much technical jargon. The following is a list of questions that you should be prepared to answer during a medical assessment:

- When/where did the incident occur? How many people were involved?
- What agent was involved? Are there distinct characteristics of the strain used that would affect antibiotic resistance, pathogenicity, or transmissibility of the pathogen?
- What was the route of exposure?
- Is there concern regarding human to human transmission of disease?
- Are there health-related risk factors associated with the individual?
- What first aid was performed? Was immediate medical care sought?
- Is the individual showing any symptoms?
- Was an animal involved and, if so, has a veterinarian been contacted to determine the health status of the animal?

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Unless you are medically qualified, don't attempt to make a medical assessment on your own. This should be done by a licensed professional. Even if you are uncertain if an actual exposure has occurred, the described steps should always be taken. It's better to err on the side of caution than to put your or someone else's health at risk. If you have any concerns or questions about this process or you question whether medical attention is necessary you can discuss the situation with the Occupational Health Program.

OBS also must be contacted in the event of biological exposures. OBS can help with any post-incident review to determine if any procedural changes are advisable. Additionally, certain incidents require notification of state and federal agencies.

Speaking of reporting, always fill out an accident report for the event. This will facilitate resolution of issues related to Workman's Compensation claims such as reimbursement of payments for prescriptions and services.

One final word of advice – Don't ever let the thought of waiting in the emergency rooms, lost work time, or issues of costs related to treatment prevent you from seeking treatment. Risks to your health should always outweigh these factors.

Describing Use of Hazardous Chemicals in Biosafety Protocols

There is often uncertainty about which uses of hazardous chemicals need to be described in a biological safety protocol. This article addresses common questions on that topic received by OBS using an FAQ format.

Q: Why do I have to describe any chemical uses in the biological safety protocol? Aren't hazardous chemicals covered through Chemical Safety?

A: Many chemicals are used routinely in laboratories. Standard precautions for handling hazardous chemicals need to be outlined in your laboratory's Chemical Hygiene Plan (CHP). Contact Chemical Safety in the Environment, Health and Safety (EH&S) Department (265-5000) for assistance with developing a CHP for your laboratory. However, potentially-hazardous chemicals used to elicit a biological outcome *in vivo* (animals, plants) or *in vitro* (cell lines, tissues) need to be covered in the biosafety protocol. Formaldehyde (formalin), for example, is a hazardous volatile chemical and probable human carcinogen used to fix tissues but it does not need to be included in the biosafety protocol because its use is not intended to induce a biological outcome.

Q: What types of chemicals should be added to my biosafety protocol?

A: Potentially hazardous chemicals include carcinogens, mutagens, teratogens, systemic toxins, and chemicals toxic to specific organs such as neurotoxins, reproductive toxins, liver toxins, kidney toxins, etc.

Chemicals that have similar functional endpoints (e.g., antibiotics, anesthetics) may have very different toxicity profiles. Thus, many common antibiotics (ampicillin, kanamycin, erythromycin) do not need to be added to the biosafety protocol because they have minimal toxicity while antibiotics that are potentially toxic such as chloramphenicol (a probable human carcinogen, experimental teratogen, and agent associated with aplastic anemia) do need to be added to your protocol.

Handling volatile hazardous chemicals heightens risks because of elevated chances of exposure. One of the precautions that is especially important for handling of volatile hazardous chemicals and that needs to be described in the biosafety protocol is use of containment. Volatile anesthetics such as urethane and isoflurane, for example, should be administered to animals in a fume hood or contained in some other fashion (e.g., scrubber device).

A common misconception is that drugs already prescribed for humans do not need to be included in biosafety protocols. The research situation is very different than the clinical one, however. Patients in consultation with their doctors weigh the risks of taking a drug against the benefits for whatever condition is being treated. When laboratory and animal care workers are exposed to drugs that are hazardous, they do not receive the benefits – only the drawbacks.

Therefore, drugs that fit into one of the above toxicity categories need to be described in the biosafety protocol. A clear example of this dichotomy is chemotherapeutic agents which are valuable in treating cancer but should not be handled by workers without using the appropriate precautions. Some of these agents (e.g., cyclophosphamide, 5-fluorouracil) are themselves classified as carcinogens and/or show some other type of toxicity.

Q: I don't know whether a particular chemical is considered hazardous or not. Where do I find this information?

A: Various sources provide information on the hazardous properties of chemicals, including:

- Material safety data sheets (MSDSs) must be kept on file for each chemical in the laboratory. MSDSs are useful for some applications – especially for chemicals that are acutely toxic. MSDSs may not be informative in some cases, particularly for chronic toxicity effects. For example, many MSDSs indicate “unknown” for reproductive effects. Current toxicology literature tends to be more complete.
- TOXNET (<http://toxnet.nlm.nih.gov/>) is a toxicology data network that provides information from databases such as HSDB (toxicology summaries), TOXLINE (toxicology articles), CCRIS (carcinogens), DART (developmental and reproductive toxins), and GENETOX (mutagens).
- National Toxicology Program (NTP) Report on Carcinogens gives descriptions of chemicals that are classified as known or probable human carcinogens.

Q: I already filled out an animal care protocol describing use of these chemicals. Why do I also have to complete a biosafety protocol?

A: The main focus of the animal care protocol is to ensure that animal care regulations are followed. The main focus of the biosafety protocol is to ensure safety of staff and protection of the environment.

Q: Do I need to add chemicals to my biosafety protocol when I already have similar chemicals listed?

A: It depends on whether the risks might be altered.

If the protocol already covers chemicals with similar toxicity profiles and the chemical added will be used in similar ways (e.g., dose, route of administration, type of animal receiving treatment, etc. are the same), an amended protocol does not need to be submitted.

If risks might be altered with the added chemical, the protocol should be amended. An obvious example of a change that alters risk is use of higher doses. Another factor that can change the risk assessment is route of administration. Chemicals administered in food or water or topically are more difficult to contain than those that are injected. Precautions for preparation of food and drinking water and for handling animals and animal wastes need to be tailored to the administration route. A third example of an aspect that can alter risk is the species of animal. The size of animal, animal behaviors, and how that species metabolizes the chemical are considered since these characteristics affect associated risks.

Q: What sections of the biosafety protocol do I need to complete when amending it for use of chemicals?

A: The main sections to revise are Section IV.E (table specifying chemicals and how they are used), Section V.A (brief description of design and objectives), and Section V.C (safety precautions), particularly the Hazardous Chemical column in the Section V.C.g table. There may be other sections that need to be updated such as Section II if adding locations or Section IV.D if adding animals.

Q: What precautions should be used with hazardous chemicals?

A: Precautions for use of hazardous chemicals in laboratories and animal facilities are outlined in a Chemical Handling Memo which is available from our website and include use of appropriate personal protective equipment (PPE), containment, hazard signage, and training.

Q: What level of detail is needed for completing the biosafety protocol?

A: Provide sufficient information so a risk assessment can be done. If a dose is yet to be determined, give an upper estimate of dose to be used. If uncertain about the toxicity of chemicals, precautions to be taken, or how to complete the biosafety protocol, feel free to contact OBS and/or the EH&S Department for more information.

Tailor Precautions to the Specific Hazards

Risk assessment involves evaluation of factors such as what hazardous materials are handled and what procedures are performed in order to determine what precautions are appropriate. A “one size fits all” approach rarely works well. Instead, safety measures must be optimized to the specific circumstances.

For example, biosafety level 2 (BSL-2) precautions are used for a range of materials and procedures. BSL-2 conditions are appropriate for potentially infectious materials such as primary and established human and Old World monkey cells because of the possibility that these cells carry pathogens. Likewise, administering potentially infectious material to immunocompromised animals is done using BSL-2 precautions because of the potential for any pathogens present to propagate and to spread to staff or other animals. Across the BSL-2 continuum is a wide variety of risk group 2 pathogens.

There is a basic set of precautions that are either required or recommended for BSL-2 activities but the set of precautions also needs to be customized to the specific situation. Biosafety in Microbiological and Biomedical Laboratories (BMBL) and the NIH Guidelines for Research Involving Recombinant DNA Molecules are two valuable references that can be accessed from our website. Some of the factors to include in a risk assessment of BSL-2 projects are:

- Type of pathogen - Pathogen traits vary tremendously between different types of pathogens and can differ significantly even among different strains of the same pathogen. This variability often necessitates use of modified precautions.
- Route of transmission - Aerosol transmission increases the potential risk. Thus, aerosol-transmitted pathogens need to be handled in containment (e.g., a biosafety cabinet) on a routine basis while pathogens that are not normally aerosol-transmitted need to be handled in containment only when doing aerosol-generating activities.

- Aerosol-generating activities - A few examples of common activities that generate aerosols are centrifugation, pipetting, and cage changes of animals. Use of containment equipment (or respiratory protection for procedures that cannot be done in containment) is appropriate for activities with hazardous materials that can generate aerosols.
- Quantity and concentration of pathogen - Use of large volumes and/or high concentrations of pathogens will increase risk so that more stringent precautions are required.
- Disinfection - Different types of disinfectants are suitable for different types of pathogens. Ensuring that the disinfection/disposal method actually inactivates the particular pathogen(s) is imperative for protecting staff and the environment.
- Laboratory versus animal research - Although there are many similarities in BSL-2 conditions in laboratory and animal facilities, animal research adds some complicating factors that require modified precautions that protect staff in animal handling situations.
- Other hazardous materials - Often research protocols involve hazardous materials other than biological ones such as radioactive materials and/or hazardous chemicals. Precautions need to be implemented to protect against all types of hazard present.

Using precautions tailored to the specifics of your work environment is the best way to protect you, other staff, and the environment. Contact OBS for assistance in doing risk assessments and adapting precautions to your particular work situation.

Protocol Form Improvements

The biosafety protocol not only forms the basis of an independent risk assessment but also serves as a document for training staff in appropriate precautions. A copy of the registered protocol should be available in the lab for personnel to review. The protocol form has recently undergone several subtle yet significant changes designed to clarify the information that needs to be included. The revised form is available from the OBS website. It is important to use the latest version of the form when preparing a new protocol or an update (3-year renewal) to avoid being asked to provide additional information. The following is an overview of the changes in the form:

1. Material Transfer Agreements - MTAs are tracked in a manner similar to funding for proposals and awards. The title and source are entered in the lower area of the Core Registration Information (Section I). Furthermore, if the MTA involves material that relates to an aspect of a project that is not yet described in an existing protocol, additional detail is needed in the appropriate sections, such as the Design and Objectives (Section V.A).
2. NIH Guidelines Assessment – Some protocols involve activities that are subject to the NIH *Guidelines for Activities Involving Recombinant DNA Molecules*. Section IV.A(iii) has been revised to have columns for the specific information that is sought. Below are some examples for entries. This exercise can be difficult, yet is required by NIH. Please feel free to consult with OBS staff for assistance with this determination.

Vector or rDNA element and activity	Biosafety Level	Guidelines citation
Clone genes in <i>E. coli</i> K-12 strain	BSL-1	III-F; App.C
Clone genes in <i>E. coli</i> BL21 strain	BSL-1	III-E
Large scale production of protein in <i>S. pichia</i>	GLSP*	III-D-6; App.K
Introduce fluorescent marker trait into <i>Salmonella</i>	BSL-2	III-D-1
Transfect plasmids to silence genes in frog embryos	BSL-1	III-E
Administer recombinant adenovirus to mouse cells	BSL-2	III-D
Handle recombinant amphotrophic Moloney leukemia virus	BSL-2	App.B-V
Clone human genes into established human cells	BSL-2	III-E
Generate knockout strain of mice	BSL-1	III-D
Test gene-deleted rhinovirus as vaccine in animal	BSL-2	III-D

*GLSP – Good Large Scale Practices

3. Biosafety Level for Microbes - The section for listing the microorganisms (IV.B) has a new column for entering the appropriate risk group or biosafety level appropriate for the microorganism. A good source for this information, when in doubt, is Appendix B of the NIH Guidelines, which provides definitions for the risk groups and listings for RG2-RG4 pathogens. Take note that the lists are incomplete and have not been updated for

several years. Furthermore, some exotic microbes may be subject to a higher level of containment than indigenous strains of the same organism.

4. Statement Regarding Human Embryonic Stem Cells

Animals that have received human embryonic stem cells may not be bred. This compliance point has been added to Section IV.G. Investigators who do this type of experiment are asked to confirm that they are aware of the restriction and will not breed the animals. If you transplant human embryonic stem cells into an animal, you will need to fill out this section.

Dual Use Research of Concern

The outcome of legitimate research in the biological sciences typically has benefits for mankind, but also has potential to be used for nefarious purposes with serious consequences for public health or the environment. The National Science Advisory Board of Biosecurity (NSABB) has been established to provide advice to federal agencies on ways to minimize the potential public health and national security threats that may result from the misuse of information and technologies resulting from such dual use research. One of NSABB's major tasks is to recommend an oversight process to manage research information with dual use potential. Exemplary of research of concern was the 2001 Australian report on genetically modified mousepox virus, research intending to develop a contraceptive vaccine to control rodent populations, but resulting in unanticipated enhanced lethality of an otherwise mild virus.

An NSABB draft report* provides an overview of the issues and proposes a plan for oversight that relies heavily on local management at the institutions where this research is conducted. The proposed framework is built upon investigator awareness, peer review, local institutional responsibility, and public input. The vital role of life sciences research in public health and other aspects of national security is recognized, as is the need to avoid impeding progress of such research. A beneficial result would be that scientists demonstrate that they take responsibility for the dual use potential of their work.

Since much of life science research can be defined as dual use, the NSABB has focused its efforts on "dual use research of concern". This is defined as, "Research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agriculture, plants, animals, the environment, or material." The draft proposal provides an extensive list of criteria for identifying research of concern, such as:

- increasing virulence or host range of a pathogen;
- reducing susceptibility of a pathogen to a treatment;
- increasing susceptibility of a host to a pathogen;
- facilitating the ability of an agent or toxin to evade detection;
- reconstructing an eradicated or extinct biological agent (e.g., smallpox).

The proposed framework of oversight involves mandatory annual training and assurances that researchers are assessing their research for dual use potential. The proposal recommends evaluation of research for its dual use potential at its inception and then periodically. Once the subset of "dual use research of concern" is identified, all related communications would be screened, including grant proposals, seminar presentations, posters, and publications. A committee, similar to the Institutional Biosafety Committee, would provide peer review.

Procedures to mitigate the impact of release of information are detailed. Institutions would have a plan for responsible communication of dual use research information, which would extend to public understanding of, and appreciation for, the research effort. Communications of research deemed to have a high potential risk of misuse would involve federal intervention.

Significant concerns have been expressed about the proposed framework. The determination that a research project meets the definition of dual use research of concern involves a personal judgment and interpretation, imaginative thinking, and a wide range of technical competence, with likely inconsistencies between institutions. The proposed framework focuses on federally funded research, and depends on voluntary compliance by other entities (e.g., industry). Also, the framework does not address the international flow of scientific information. The proposed strategy recommends development of an enforcement mechanism, including penalties for noncompliance such as making compliance a term and condition of funding, which may cause institutions to take a conservative approach to implementing oversight.

The proposed oversight mechanisms, if adopted, will have far-reaching consequences, so it behooves affected individuals to become knowledgeable and to voice their opinions before the federal requirements are promulgated. The outcome of the discussion of dual use research of concern will hopefully be a reasonably balanced oversight system, enabling vital research to go forward, allowing open communication of research results, and maintaining public confidence in the conduct of research. More information about NSABB is posted at www.biosecurityboard.gov.

**Proposed strategies for minimizing the potential misuse of life sciences research. Draft document of a Working Group of the NSABB, April 2007.*

Safety Products/New Gadgets - Homogenizer

Homogenization is an activity that commonly produces aerosols. A new homogenizer is available that has sealed containers. The SilentCrusher S from the Heidolph Company has variable speed control from 15,000 to 75,000 rpm. It weighs just 1.75 lbs and has a relatively low noise level (54 dB max). The sample container is held to the unit by O-rings that prevent release of aerosols. The homogenizing tool is suspended to give ample room for an ice bath to surround the sample container for specimen cooling. There is no fan used because this device uses a magnetic drive-less system. The three easy-clean, autoclavable, homogenizing tools are available, for microfuge tubes and small and large test-tubes. Contact your Fisher representative for details. This information is provided as a service to UW staff and students and is not an endorsement of particular products or vendors.

Classes Offered by OBS

Shipping Infectious Substance and Other Biological Materials; Packaging Workshop

- **Class: Wednesday October 17, 2007** 1:00 – 3:30 p.m. at Union South
Optional Workshop: Wednesday October 17, 2007 3:30 – 4:30 p.m. at Union South
- **Coming soon:** A web training module for shipping dry ice as the only dangerous good.

Basic Biosafety

- **Thursday November 8, 2007** 1:30 – 4:30 p.m. at 1345 Health Sciences Learning Center

Advanced Biosafety

- **Wednesday November 14, 2007** 1:30 - 4:00 p.m. at Union South

Registration is required for these courses.

Contact OBS at 263-2037 or biosafety@fpm.wisc.edu for more detailed information.

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