BioSide Lines

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The Newsletter of the Office of Biological Safety, UW-Madison Safety Department
www.fpm.wisc.edu/biosafety

Safety and TAT Fusion Proteins

Proteins and peptides fused to protein transduction domains (PTD) such as TAT enable fusion proteins to readily cross cell membranes. TAT fusion proteins are becoming a widely-used tool to deliver biologically active macromolecules into a variety of cell types *in vitro* and *in vivo*. PTD are short peptides that are highly enriched in basic amino acids. The TAT PTD sequence (typically encoding 9 amino acids) is derived from a human immunodeficiency virus (HIV) gene, but the TAT PTD peptide presents no infectious risk.

TAT fusion proteins may be hazardous because they bypass the normal regulation of entry into mammalian cells and lack specificity for host or eukaryotic cell type. The level of hazard is very dependent on the peptide or protein attached to the PTD (e.g., an oncoprotein would pose a higher hazard than a housekeeping protein). Other risk factors include quantities handled, turnover times, and whether aerosol-generating steps are done. Information on turnover time is often not available for many TAT fusion proteins. The highest risk would be from exposure of personnel to aerosols generated by activities with concentrated TAT fusion proteins.

A common lab activity that produces aerosols is sonication. When TAT fusion proteins are produced in bacteria, sonication is routinely used to lyse the bacterial cell wall. This aerosol-generating step can be avoided by producing TAT fusion proteins in mammalian expression systems which do not require sonication. Fusion proteins are further purified prior to transducing the proteins into cell lines or animals.

Standard precautions for handling TAT fusion proteins should include wearing appropriate personal protective equipment (PPE) such as lab coats, gloves, and protective eyewear and doing aerosol-generating activities such as sonication in containment (e.g., biosafety cabinet, fume hood). As always, staff should be trained regarding hazards presented by the material.

Containment equipment should be used while administering TAT fusion proteins to animals if aerosols could be generated in the process, such as through use of a needle and syringe. After TAT fusion proteins are administered to animals, there are no significant hazards present and handling of these animals can be safely performed using animal biosafety
level 1 precautions.

Ductless Fume Hoods: Not Acceptable

The Safety Department strongly discourages the use of ductless fume hoods and, except in extremely rare circumstances, will not approve their purchase for use in labs. The hoods generally do not provide reliable protection against chemical exposures and may, in fact, give workers a false sense of security.

Ductless fume hoods are designed to remove hazardous fumes and vapors from the work area. A ductless hood is an open faced enclosure designed to protect the user from laboratory and industrial airborne contaminants. Its intended function is similar to a laboratory fume hood, but it depends on filters to trap the contaminants and is not connected to a ducted exhaust system. The air passes through an adsorbent such as activated charcoal and then recirculates back into the room. The objective of the filtration system is to reduce the exhausted levels of air contamination below the acceptable TLV (threshold limit value, an air quality standard).

The system's appeal is largely economic because it does not require the expensive ductwork and fan systems that traditional hoods need to exhaust fumes to the outside. In practice, however, ductless fume hoods are the

source of numerous problems. The most critical issue is that they often do not provide adequate protection against the variety of chemicals used in a typical university laboratory since the filtration system is designed for specific chemicals and is limited in capacity. Ductless fume hoods often do not provide an adequate face velocity, allowing the hazardous vapors to waft into the room. Furthermore, the filters cannot be monitored appropriately for efficacy of capture and once saturated cannot capture additional chemicals. They are also plagued with problems associated with "breakthrough" and release of chemical vapors from the filter. Most ductless fume hoods are inherently unsafe for use with flammable liquids.

These ductless fume hoods are expensive for the user to maintain. Used filters may be classified as hazardous waste resulting in a disposal fee of several hundred dollars each. Annual maintenance costs to the owner could exceed $500 depending on the use (amount and type of chemical). Unlike traditional vented fume hoods which the Physical Plant inspects, repairs if necessary, and certifies annually, the university does not provide services to maintain ductless fume hoods.

When ductless fume hoods are used, it is the supervisor's responsibility to clearly label and inform affected individuals that this device provides limited protection from hazardous chemicals. The Chemical Hygiene Plan must cover its use including details about the hazardous chemical, the expected filter capacity and loading rate, a plan for filter replacement and disposal, inspections, and annual certification.

Please feel free to contact Darren Berger (263-2187) or Jack Wunder (262-0490) for further discussion of ductless fume hoods.

Respirator Fit Testing

Annual fit-testing is a requirement of law for all personnel who are required by their employer to wear N95 masks or respirators on the job. The Safety Department is pleased to announce the purchase of a TSI PortaCount Plus with N95 Companion to fit-test your respirators and N95 masks. This $13,000.00 instrument is a computerized state of the art method of fit-testing that eliminates guesswork and chemical exposure. The PortaCount can be used to fit test almost any tight-fitting respirator; half and full face, powered air purifying, SCBA's, plus N95 and other disposable filtering facemasks.

The PortaCount determines the difference between the concentration of microscopic particles that exist in ambient air and the concentration of those particles that leak into the respirator while you simultaneously perform dynamic moving and breathing exercises intended to stress the respirator seal. The ratio of these two numbers is the fit factor. A fit factor of 100 for example, means that the air inside the respirator is 100 times cleaner than the air outside the respirator.

Respirator training and fit-testing are free services that the Safety Department offers UW Madison employees. If you have been medically cleared to wear a mask or respirator, please contact Bill Deppen at 262-9179 to arrange fit-testing. If you need to obtain medical clearance, please contact Tom Kenney, RN at 263-2177.

The Export License Requirement

Heads Up! Be sure to check the Commerce Control List before you send microorganisms, toxins or their genetic elements to another country. The U.S. Department of Commerce requires a license to ship certain materials to other countries, even though the country may be an ally and/or the pathogen may be endemic there. The lists of regulated materials and destinations are somewhat eclectic and not static.

The Commerce Control List, part of the Export Administration Regulations, can be found at: *www.access.gpo.gov/bis/ear/pdf/ccl1.pdf*. The following sections are of particular relevance to biological researchers.

Human and zoonotic pathogens and toxins including subunits - 1C351
Animal pathogens – 1C352
Plant pathogens – 1C354
Genetic elements and genetically modified organisms - 1C353.
Vaccines, immunotoxins, medical products, diagnostic and food testing kits – 1C991

The first step is to check the appropriate lists of items. Finding your material on the list means that there is a mandatory licensing requirement for export to at least one destination. The subsequent steps to obtain a license can be complicated and you may contact OBS for assistance. The penalties for violating the export control regulations can be severe.

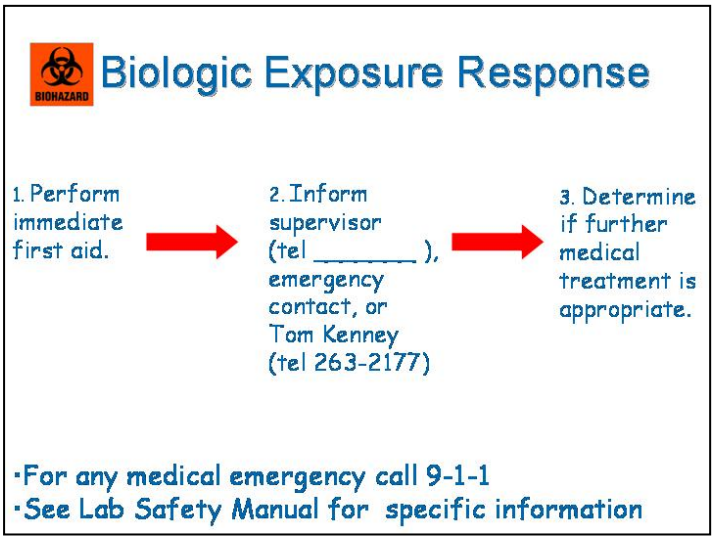
Biological Exposure Procedures

- Wash and scrub any scratch, wound, bite or needle stick for 15 minutes with soap and water.
- Use a plumbed eyewash for 15 minutes for any splash to the eyes.
- Inform your supervisor.
- Seek further medical attention or a medical evaluation at the UW Hospital, Employee Health Office, 2820 University Avenue, Monday - Friday, 8:00 am to 4:30 pm (After hours and weekends go to the UW Hospital Emergency Room).
- Contact Occupational Health at 263-2177

A poster and procedure template are provided at the OBS website to assist researchers in responding quickly to a laboratory accident involving a potential exposure to a biological agent. A copy of the completed template, Biological Exposure Procedure and Specific Biological Agent Information, should accompany the individuals when they seek medical attention for an exposure. This will assist medical personnel in evaluating the incident and providing appropriate treating.

Biological Exposure Procedure and Specific Biological Agent Information is a template to be modified with agent specific information. The form serves to organize important information about the agent(s) to which personnel might be exposed and the appropriate incident response. It is important to include strain specific information, particularly with regard to atypical antibiotic resistance of the laboratory strains.

The Biologic Exposure Response poster (PowerPoint) should be posted in a highly visible location within the lab. Enter the supervisor's telephone number and then use a color printer to produce the poster.



Poster

Both documents are useful for training personnel on incident response. With minor modifications, the template also could be applied to other hazardous materials.

Employees are reminded of their obligation to complete the Employee's Work Injury and Illness Report, available from your supervisor or electronically at the following URL, www.bussvc.wisc.edu/ecbs/uwempl-wkc-menu.html.

BIOLOGICAL EXPOSURE PROCEDURE
AND SPECIFIC BIOLOGICAL AGENT INFORMATION

In case of an exposure, bring this procedure and a copy of the attached material safety data sheet (MSDS) with you if further medical care is required.

Principal Investigator_____Date prepared_____
Building_____ Room(s)_____
Emergency contacts:

	Name	Work phone	Home phone	Cell phone
1.				
2.				
3.				
4.				

Biohazardous agent(s):

	Name	Strain	Drug resistance traits
1.			
2.			
3.			
4.			

Please attach appropriate MSDS or other agent and strain specific information. The Health Canada website at address http://www.phac-aspc.gc.ca/msds-ftss/index.html may serve as one starting point for this information.

In the event of a biological exposure, these steps should be followed:

1. Immediately clean the wound, bite, scrape, scratch, needlestick or other injury for 15 minutes with antiseptic soap and water. Gently squeeze the area to encourage bleeding if possible. Cover the area with a sterile dressing or apply a bandage. First aid supplies are located _____
   For a splash to the eyes, use an eyewash for 15 minutes. The nearest eyewash is located_____
   If someone has a possible or definitive inhalation or ingestion exposure, proceed to step 2. Telephone 9-1-1 for a severe wound or substantial bleeding, any serious medical situation, and any exposure or health situation you are uncomfortable managing on your own
2. Immediately contact your principal investigator, supervisor, or any other emergency contact as soon as possible after the incident. If available, the occupational health officer Tom Kenney RN can also serve as a contact person. He can be reached at
   263-2177(office) or 516-1925(cell).
3. If further medical care is required, proceed to either University Hospital Employee Health Service or the University Hospital Emergency Services Department for a medical assessment and treatment. Employee Health Service is available Monday-Friday from 7:00am until 4:30pm. For after hours and weekends, go to the University Hospital Emergency Services Department. If there are any concerns regarding disease transmission go only to the Emergency Services Department.

Note: The complete template and poster are available at the OBS website, Emergency Procedures.

Shipping Infectious Substance and Other Biological Materials

The Office of Biological Safety will provide training and certification for shipping Infectious Substance and other biological materials, with a focus on safety and regulatory compliance for research laboratories. The Department of Transportation requires that persons involved in shipping hazardous materials in commerce be trained and certified in proper handling of these materials.

Tuesday, July 12, 2005
Union South 1:30 – 4:00 p.m.
Refreshments will be served.

Registration is required. Contact OBS at 263-2037 or biosafety@fpm.wisc.edu.

All staff are welcome to attend this class for initial training or re-certification. Staff approaching their two-year expiration for certification will receive a notice in advance of that date. Computer-based training is available only for those who attended the class for their initial certification.

Basic Biosafety Class Offered

This class will give an overview of basic biological safety. Topics include: biosafety levels and biohazard containment, good microbiological techniques, waste disposal, risk assessment, and emergency preparedness. It is intended primarily for students and staff who are new to this institution and/or new to working with biological materials in a laboratory. Everyone is welcome to attend.

Tuesday, July 19, 2005
Union South 1:30 – 3:30 p.m.

Registration is required. Contact OBS at 263-2037 or biosafety@fpm.wisc.edu.

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