Laboratory Biosafety Manual

North Carolina State University

Environmental Health & Safety Center
2620 Wolf Village Way
Raleigh, NC 27695

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Chapter 1:

Procedures Governing the Use of Biohazardous Agents

This Biosafety Manual provides a guide to common practices related to working with biological materials at North Carolina State University. This Chapter provides procedures governing the registration and procurement of biological materials at NC State. Subsequent chapters provide a review of pertinent federal and state government regulations, information about training, safe work practices, safety equipment, and personal protective equipment.

Biohazardous agents, or "biohazards", are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. The risk can be direct through infection or indirect through damage to the environment.

Biological materials that investigators may not consider to be biohazardous may still be regulated under federal, state, or local statutes and guidelines as biohazardous materials. Therefore NC State requires the following of investigators using any of the biological materials listed below:

1. Investigators must obtain approval from the Institutional Biosafety Committee (IBC) of the following biological materials prior to the procurement of the materials necessary to initiate the project:

    - recombinant DNA in organisms,
    - creation of transgenic plants or animals,
    - human and other primate-derived substances (blood, body fluids, cell lines or tissues),
    - organisms and viruses infectious to humans, animals or plants (e.g. parasites, viruses, bacteria, fungi, prions, rickettsia);
    - biologically active agents (i.e. toxins, allergens, venoms) that may cause disease in other living organisms or cause significant impact to the environment or community.

2. Investigators must procure all of the biological materials listed above through the MarketPlace online procurement process (This site contains the procurement process for suppliers not presently listed in the MarketPlace).

Investigators register projects with the IBC by completing the registration form indicated below. Laboratories with work practices alternative to this Biosafety Manual should include SOPs for such practices with their registration form. All registration documents for use of biologicals should be stored with the Safety Plan and Supervisor Checklist.

Registration Form for Use of Biological Materials at NC State

To register your biological materials, complete a Biological Use Authorization (BUA) form and submit it to the University Biosafety Officer (BSO) at Environmental Health and Safety as indicated in the instructions on the form. The BSO will forward your form to

the Institutional Biosafety Committee (IBC) for pre-review and may contact you with questions or concerns about your proposal (e.g. documentation, lab practices, containment, training, equipment, personal protective equipment, facilities, etc.). The IBC reviews registrations at regularly scheduled meetings.

The Exposure Control Plan for Bloodborne Pathogens

Any research with material that was derived from humans including blood, body fluids, tissues, primary or established cell lines requires the PI to indicate "Bloodborne Pathogens" on their Safety Plan and complete the appropriate section of the BUA. In addition, an Exposure Control Plan must also be adopted to meet OSHA regulation 1910.1030 for Bloodborne Pathogens in the workplace. The Exposure Control Plan must be updated annually and according to the instructions on the form. For more information, refer to the EH&S website for Bloodborne Pathogens.

The Safety Plan

Each BUA lists its associated Safety Plan number. Availability of biological safety cabinets and autoclaves are indicated on the PI's Safety Plan. Registration documents may be uploaded to the Safety Plan but this does not constitute approval of the BUA by the IBC. The BUA is completed aside from the Safety Plan and submitted to the Biological Safety Officer.

The BSL-2 Checklist

In 2007 the CDC enhanced requirements for all work at BSL-2. To ensure laboratories meet basic requirements at the federal, state, and local levels for BSL-2 practices and containment, the BSL-2 checklist is completed per the instructions on the form.

Chapter 2: NIH Recombinant DNA Guidelines

The NIH Guidelines for Research Involving Recombinant DNA Molecules (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html) detail procedures for the containment of rDNA research. These Guidelines apply to all institutions that receive NIH funding for rDNA research. All Investigators at the institution must comply with the Guidelines even if their individual research is not funded by NIH. Consequences of noncompliance include suspension, limitation, or termination of NIH funds for rDNA research at the institution, or a requirement for prior NIH approval of rDNA projects at the institution.

The original guidelines were issued in 1976 due to public concern for safety, environmental impact, and ethical implications of rDNA research. The purpose of the guidelines is to specify safe handling practices and containment levels for rDNA molecules, organisms and viruses containing rDNA molecules, and transgenic animals and plants.

Responsibilities under the Guidelines:

1. The Institution must:
    a. establish an Institutional Biosafety Committee (IBC);
    b. ensure compliance with the NIH Guidelines by investigators;
    c. appoint a Biological Safety Officer if rDNA > 10 L or at BSL-3;
    d. report any significant problems, violations or significant research-related accidents or illnesses to NIH within 30 days.

2. The IBC must:
    a. review, approve and oversee rDNA research to ensure compliance with the Guidelines;
    b. determine necessity of health surveillance of personnel;
    c. ensure training for IBC members, staff, PIs, and laboratory staff;
    d. set biosafety containment levels.

3. The Principal Investigator must:
    a. be proficient in good microbiological techniques;
    b. supervise staff to ensure safety practices are followed;
    c. instruct laboratory staff on:
        i. the risk of agents used in the lab,
        ii. safe work practices,
        iii. emergency procedures for spills and exposures,
        iv. the reasons for vaccinations and serum collection, when applicable;
    d. ensure that:
        i. proper biosafety, biowaste, and shipping procedures are followed by staff,
        ii. SOPs are developed and followed for spills, exposure, loss of containment, and reporting research-related accidents and illnesses,
        iii. women of child-bearing age are provided with information regarding immune competence and conditions that may predispose them to infection,
        iv. biological containment is maintained,
        v. unsafe work errors are corrected;
    e. determine whether their research is subject to Section III-A, B, C, D or E of the Guidelines;
    f. propose containment levels in accordance with NIH Guidelines and a risk assessment;
    g. submit to the IBC for approval before initiating research (Biological Use Authorization);
    h. notify the EH&S Biosafety Officer of:
        i. changes to research before modifications are implemented
        ii. any significant research-related accidents and illnesses
        iii. any significant problems with containment procedures
        iv. violations of NIH guidelines.

4. Environmental Health & Safety must:
    a. conduct lab inspections;
    b. develop emergency and reporting procedures;
    c. investigate lab accidents;

d. report rDNA incidents, violations of the Guidelines to IBC;
e. provide general biosafety training.

5. The NIH Office of Biotechnology Activities (OBA) must:
    a. manage the Recombinant DNA Advisory Committee (RAC)
    b. conduct trainings of IBCs
    c. review human gene transfer protocols
    d. review the following rDNA experiments:
        i. deliberate transfer of drug resistance that could compromise disease control
        ii. cloning toxins with LD50 < 100 ng/Kg body weight
        iii. rDNA experiments involving restricted agents
        iv. use of restricted poxviruses in presence of helper virus

Classification of rDNA research:

To determine whether your research is subject to Section III-A, B, C, D, E, or F please refer to the Classification Page at http://www.ncsu.edu/ncsu/ehs/www99/left/bioSafe/forms/Forma2.pdf . A summary of these classifications is provided here.

Research that will require review by the IBC and the NIH prior to initiation:

Experiment	Section
Transfer of drug resistance that affects disease control	III-A
Cloning toxin molecules with LD50 <100 ng/Kg body weight	III-B
Transfer of rDNA into human subjects	III-C

Research at NC State involving rDNA molecules that will require review by the IBC prior to initiation:

Experiment	NIH Section
Experiments using Risk Group 2, 3, or 4 agents as host-vector systems	III-D
Experiments in which DNA from Risk Group 2, 3, or 4 agents is cloned into non-pathogenic prokaryotic or lower eukaryotic host-vector systems.	
Experiments involving the use of recombinant or reassortant viruses in tissue culture systems; or defective recombinant viruses in the presence of helper virus or packaging cells in tissue culture systems (this includes all eukaryotic viruses.	
Experiments that generate transgenic animals, including insects (with the exception of transgenic rodents requiring BSL-1 containment).	
Experiments involving viable rDNA-modified microorganisms tested on whole animals.	
Experiments involving whole plants that require BSL-3 containment.	
Experiments involving more than 10 liters of culture.	
Experiments involving human influenza strains H2N2, 1918 N1N1, and/or highly pathogenic H5N1.	
Introduction into cultured cells of any rDNA containing greater than half but less than 2/3 of a eukaryotic viral genome (with the exception of Risk Group 3 or 4 agents)	III-E
Cloning in non-pathogenic prokaryotes and non-pathogenic lower eukaryotes.	
Generation by embryo injection of transgenic rodents requiring BSL-1 containment.	
Breeding 2 different transgenic strains of rodents to generate novel transgenic strains requiring BSL-1 containment.	

Experiments involving whole plants that require BSL-1 or BSL-1 containment.	
Experiments not specified on this sheet.	
Cloning of DNA for more than one-half of the genome of RG1 or RG2 human or animal pathogens, or cloning of known oncogenes.	Apdx C

Risk Assessment and Containment Level

A risk assessment must be conducted to determine the appropriate Biosafety Level (BSL1-BSL4) of the agent used in your research. Biosafety Levels for rDNA research with whole animals, plants, and volumes greater than 10 liters have specific containment and reporting requirements. Contact the Biosafety Officer for help in conducting your risk assessment.

1. Determine the NIH Risk Group (RG) of the agent – Appendix B of the Guidelines.
2. Evaluate the following characteristics of the agent: virulence, pathogenicity, infectious dose, environmental stability, exposure route, communicability, volume/concentration, and availability of vaccine or treatment.
3. Evaluate the gene product for toxicity, allergenicity, activity, e.g. oncogenic.
4. Determine the level of containment necessary (Level 1, 2, or 3). NC State does not have a Level 4 containment facility.

Incident Reporting to NIH

The following incidents must be reported to NIH OBA within 30 days:

1. Any significant problems or violations of the NIH Guidelines, e.g. failure to adhere to the containment and biosafety practices in the Guidelines;
2. Any significant research-related accidents and illnesses, e.g. spill or accident leading to personal injury or illness or a breach in containment, e.g. escape or improper disposition of a transgenic animal.

The following incidents require immediate reporting to NIH OBA:

1. Spills or accidents involving rDNA requiring BSL2 containment resulting in an overt exposure, e.g. needlestick; splash in eyes, nose, mouth; or accidental aerosolization/inhalation;
2. Spills or accidents involving rDNA requiring BSL3 containment resulting in an overt exposure or potential exposure, e.g. spills of high risk recombinant materials occurring outside of a biosafety cabinet.

Minor spills of low-risk agents, contained and properly disinfected, generally don't need to be reported- consult NIH OBA if uncertain. The incident report to NIH OBA can be submitted by the Institution, IBC, BSO, or PI. The report should include the response made to mitigate the problem and preclude its reoccurrence

NIH Guidelines & Appendices

NIH Guidelines Sections I-V

Appendix A Exemptions: Natural Exchangers

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Appendix P Physical and Biological Containment: Plants

Appendix Q Physical and Biological Containment: Animals

Chapter 3: Risk Groups and Biosafety Levels

The NIH classifies biological agents into risk groups based on their relative hazard in Appendix B of the NIH Guidelines. The list is not all-inclusive, but it does provide a list of more commonly encountered agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans.

Summary of Risk Groups (RG)

RG1	Agent not associated with disease in healthy adult humans; *B. subtilis, E. coli K-12,* AAV, ecotropic avian sarcoma virus
RG2	Associated with human disease which is rarely serious and preventive or therapeutic interventions are often available; Human adenoviruses, human herpesviruses (except herpes B), *Staphylococcus aureus,* amphotropic murine leukemia virus, influenza viruses type A, B, and C
RG3	Serious or lethal human disease; preventive or therapeutic interventions may be available; *Mycobacterium tuberculosis,* VEE, *Francisella tularensis*
RG4	Serious or lethal human disease; preventive or therapeutic interventions are usually not available; Ebola, Marburg, Lassa, and Herpes B virus

Resources for assigning risk group/biosafety level

Based on a risk assessment and review of the above sources, the PI will propose a biosafety level that the IBC will evaluate at the time of registration.

1. The NIH Guidelines Appendix B assigns risk groups to some biological agents.
2. The BMBL provides Agent Summary Statements that indicate the appropriate biosafety level for some infectious agents.
3. The American Biological Safety Association (ABSA) website provides a searchable database of biological agents and their assigned biosafety levels by country.

Human blood, blood products, body fluids, tissues, and cells

Biosafety level 2 practices and containment must be followed when handling human materials that may contain bloodborne pathogens, e.g. HBV, HCV and HIV. The OSHA Bloodborne Pathogens (BBP) Standard (29 CFR 1910.1030) applies to all occupational exposure to blood or other potentially infectious materials. Under the OSHA BBP Standard Departments and/or Principal Investigators are required to (1) develop a written Exposure Control Plan,(2) offer employees the hepatitis B vaccination, and (3) provide initial and annual BBP training. For more information on the impact of the OSHA BBP standard on the laboratory setting at NC State, refer to your department's or laboratory's Exposure Control Plan and the Biosafety website for Bloodborne Pathogens.

Since the mid 1990's OSHA's position has been that workers handling human cell cultures (primary or established) fall under the purview of the Bloodborne Pathogen (BBP) Standard. For more information, review the OSHA interpretation letter on the applicability of 1910.1030 to established human cell lines (06/21/1994),

Cultured cells and tissue

Cultured cells which are known to contain or be contaminated with a biohazardous agent (e.g. bacteria or viral) are classified in the same biosafety level as the agent. Cell lines that are not human or other primate cells and which do not contain known human or animal pathogens are designated biosafety level 1.

The following cells and tissue must be listed on the Biological Use Authorization form and handled at BSL2.

- Human and non-human primate primary cells, established cell lines, and unfixed tissue;
- Cell lines exposed to or transformed by a human or primate oncogenic virus;
- Cells, cell lines or tissue infected with pathogens requiring BSL2 containment.

General Laboratory Facility Biosafety Levels

The CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Ed. outlines safe lab practices, lab facilities, and safety equipment for four biosafety levels that provide appropriate containment based upon a proper risk assessment for manipulations that begins with the various risk group agents (RG1-RG4) designated by the NIH. The BMBL also describes animal biosafety levels for the use of research animals. The summary tables below were adapted from BMBL (5th Edition) and include NC State practices.

This table summarizes the biosafety levels and requirements for laboratory work at NC State.

BSL	AGENT	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to cause disease	Standard Microbiological Practices	Gloves, lab coat, eye protection, and proper footwear.	Handwashing sink, safety shower/eyewash and, autoclave required
2	Primarily by percutaneous injury, ingestion, mucous membrane exposure. Consider aerosolization.	BSL-1 practice plus: • Restricted access • Biohazard signs Biosafety manual defining “sharps” precautions, biowaste practices, medical surveillance, and spill clean-up.	At a minimum, BSL-1 protection, plus: Physical containment devices used for all manipulations requiring BSL-2 (microbes, rDNA, toxins) that cause splashes or aerosols of infectious materials; Class I or II Biological Safety Cabinets	Same as BSL-1
3	Potential for aerosol transmission	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents Personal Protective Equipment: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into lab
4	NC State does not have BSL-4 facilities			

Vertebrate Animal Work Biosafety Levels

All activities that involve the use of live animals must be registered, reviewed and approved by Institutional Animal Care and Use Committee (IACUC) before the work is initiated. This table summarizes the biosafety levels for activities in which experimentally or naturally infected vertebrate animals are used.

ABSL	Routes of Transmission	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1		Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility: • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available
2	percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus primary barriers: • Containment equipment appropriate for animal species PPEs*: • Laboratory coats, gloves, face and respiratory protection as needed	ABSL-1 plus: • Autoclave available • Hand washing sink available • Mechanical cage washer recommended
3	potential for aerosol transmission	ABSL-2 practice plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: • Appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility
4	NC State does not have BSL-4 facilities			

* PPE – Personal Protective Equipment

Arthropod Containment

The Arthropod Containment Guidelines are based on recommendations of the American Society of Tropical Medicine and Hygiene and the American Committee of Medical Entomology. The document describes arthropod handling practices, safety equipment and facilities for Arthropod Containment Levels 1-4. Guidance for design, construction, maintenance and operation of facilities for containment of nonindigenous arthropod herbivores, parasitoids and predators which may be used in biological control research is provided in the USDA APHIS – PPQ Guidelines for Containment of Nonindigenous Arthropod Herbivores, Parasitoids and Predators.

Plant Work Biosafety Levels

The NIH Guidelines provide containment levels for rDNA plant work. The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. A good resource is *A Practical Guide to Containment Greenhouse Research with Transgenic Plants and Microbes* -- Website. This table summarizes the Biosafety levels for activities in which rDNA is used in whole plants.

BSL-P	RECORDS	PRACTICES	INACTIVATE/DECON	BARRIERS AND FACILITIES
1	records of rDNA experiments in progress	Standard greenhouse care and management practices, including limited access.	Inactivate organisms before disposal outside of the greenhouse facility.	• No special barrier to contain or exclude pollen, microbes, or arthropods and birds • Floors may be gravel • Windows etc. may be open for ventilation, screens recommended.
2	BSL1-P plus: Records of all organisms entering and exiting.	BSL1-P practice plus: • **Immediate reporting of spills or releases to IBC** • Arthropods contained • Biohazard or warning signs • Greenhouse practices manual	BSL1-P plus: • Consideration of decontamination run-off water	• Floors of impervious material in greenhouse • Autoclave available BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.
NC State does not have level 3 or 4 BSL-P facilities				

Select agents

Select Agents are federally regulated agents that have potential use in biological warfare. Health and Human Services (HHS) regulates select agents targeting humans, the United States Department of Agriculture (USDA) regulates select agents targeting animals, and the USDA Plant Protection and Quarantine (PPQ) regulates select agents targeting plants. Before possessing, using, sending, or receiving select agents, NC State and the Principal Investigator must register with CDC, and/or USDA to receive official authorization for each individual requesting access to select agents. Requirements include background checks on those authorized to access select agents, security plans and inventories. Immediately notify EH&S if you discover select agents in your laboratory that have not been registered.

HHS SELECT AGENTS AND TOXINS (Target humans)

Abrin
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
Cercopithecine herpesvirus 1 (Herpes B virus)
Clostridium perfringens epsilon toxin
Coccidioides immitis
Coccidioides posadasii
Conotoxins

Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus
Ebola viruses
Francisella tularensis
Lassa fever virus
Marburg virus
Monkeypox virus
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 influenza virus)
Ricin
Rickettsia prowazekii
Rickettsia rickettsii
Saxitoxin
Shiga-like ribosome inactivating proteins
Shigatoxin
South American haemorrhagic fever viruses: Flexal, Guanarito, Junin, Machupo, Sabia
Staphylococcal enterotoxins
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses: Central European Tick-borne encephalitis, Far Eastern Tick-borne encephalitis, Kyasanur Forest disease, Omsk Hemorrhagic Fever, And Russian Spring and Summer encephalitis
Variola major virus (Smallpox virus) and Variola minor virus (Alastrim)
Yersinia pestis

OVERLAP SELECT AGENTS AND TOXINS (Target humans & animals)
Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei (formerly *Pseudomonas mallei*)
Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*)
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan Equine Encephalitis virus

USDA SELECT AGENTS AND TOXINS (Target Animals)
African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (Exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Ehrlichia ruminantium (Heartwater)
Foot and mouth disease virus
Goat pox virus
Lumpy skin disease virus
Japanese encephalitis virus
Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1)
Menangle virus
Mycoplasma capricolum subspecies capripneumoniae (contagious caprine pleuropneumonia)

Mycoplasma mycoides subspecies *mycoides* small colony (*Mmm*SC) (contagious bovine pleuropneumonia)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (exotic): Indiana subtypes VSV-IN2, VSV-IN3
Virulent newcastle disease virus

USDA PPQ SELECT AGENTS AND TOXINS (Target plants)
Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly *Pyrenochaeta glycines*)
Ralstonia solanacearum race 3, biovar 2
Rathayibacter toxicus
Schlerophthora rayssiae var *zeae*
Synchytrium endobioticum
Xanthomonas oryzae
Xylella fastidiosa (citrus variegated chlorosis strain)

GENETIC ELEMENTS, RECOMBINANT NUCLEIC ACIDS, and RECOMBINANT ORGANISMS

1. Nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the select agent viruses.
2. Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed if the nucleic acids are in a vector or host chromosome and/or can be expressed *in vivo* or *in vitro*
3. Listed viruses, bacteria, fungi, and toxins that have been genetically modified.

Exclusions

1. The select agent rule does not include any select agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
2. The select agent rule does not include non-viable select agent organisms or non-functional toxins.
3. The HHS secretary may exclude attenuated strains or toxins if it is determined that they do not pose a public health threat.

Exempt Quantities of Toxins

The listed toxins are exempt from CDC and USDA registration requirements if the maximum allowable exempt quantity per Principal Investigator is not exceeded. PI's must keep toxin locked and maintain inventories to ensure maximum exempted amount is not exceeded.

Toxin	**Maximum Exempted Amount per PI**
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Clostridium perfringens epsilon toxin	100 mg
Conotoxins	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Shigatoxin	100 mg
Staphylococcal enterotoxins	5.0 mg
Tetrodotoxin (TTX)	100 mg
T-2 toxin	1000 mg

Chapter 4: Training

Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials.

Laboratory Training Requirements for Biosafety

EHS offers an introductory biosafety primer that includes much of the material in this manual and the BSL-2 Checklist. This Laboratory Biosafety Training, available from the EHS website, is *required for those who use --or supervise a laboratory that uses-- recombinant DNA molecules at BSL-1 or BSL-2 containment or any work requiring BSL-2 containment.* This Laboratory Biosafety Training is strongly recommended for those using biological materials (other than recombinant DNA molecules) at BSL-1 containment.

Following the Laboratory Biosafety Training, principal investigators are responsible to train and retrain new staff in practices to the point where aseptic techniques and safety precautions become second nature. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.

Additional Training for PI's Working with rDNA Molecules

Training on the NIH Guidelines for Research Involving Recombinant DNA Molecules is required by the NIH of all Principal Investigators with labs working with recombinant DNA molecules. While the online EHS Laboratory Biosafety Training (above) meets the minimum requirement, additional training slides are available online from the NIH website at http://oba.od.nih.gov/oba/ibc/IBC_Basics/Introduction%20to%20the%20NIH%20Guidelines%20and%20IBC%20responsibilities.pdf .

Personal Health Status

Also, personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to review the university's Reproductive Health Protection Program and Medical Surveillance Program.

Other training sessions are available from EHS at http://www.ncsu.edu/ehs/training.htm

Chapter 5: Medical Surveillance

A medical surveillance program is provided through NC State for personnel who are occupationally at risk of exposure to bloodborne pathogens (BBP), have direct contact with research animals, or receive vaccines for various infectious agents, e.g. vaccinia, rabies, measles, used in the laboratory. The bloodborne pathogens program follows the department or supervisor's Exposure Control Plan and includes hepatitis B vaccine and post-exposure evaluation and follow up at no cost to the employee.

In addition to being offered recommended vaccines, lab workers may be offered collection of baseline serum samples and/or tests as appropriate for agents handled in the lab, e.g. TB skin test. All BSL-3 laboratories are administered medical surveillance programs individually as detailed in the labs' BSL-3 manual & documentation. All medical surveillance and vaccination requirements specific to laboratory research are listed on the Biological Use Authorization for review by the IBC at the time of registration.

Chapter 6: Biosafety Cabinets and Other Safety Equipment

Biological safety cabinets (BSC) control airborne contaminants during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. The Class II BSC is the most commonly used BSC at NC State.

The table below shows the type of protection provided by common hoods used at NC State. Although both the Chemical Fume Hood (CFH) and the BSC provide worker protection by enclosing the hazardous operation, CFH's are rarely substituted because of their lack of sterility. Notice, the Clean Bench does not offer worker protection.

Types of Protection

	Worker	Product	Environment
Chemical Fume Hoods (*Protection From Vapors And Gasses*)	✓		
Biological Safety Cabinets *(Protection From Particulates)*	✓	✓	✓
Clean Benches *(No Worker Protection)*		✓	

For general information, refer to the guidance document titled *Selection, Installation and Use of Biological Safety Cabinets* by the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health at http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf .

Biological Safety Cabinets at NC State

Biological safety cabinets can only protect the worker and the experiment if they have been properly selected for the intended containment function. Questions about BSCs at NC State should be directed to the EHS Biosafety Officer at 515-6858.

1. Selection

Proper selection of the a BSC is contingent on an accurate risk assessment of the hazards inherent to the work planned in the unit (e.g. chemical, radiological, biological hazards). Selection should consider (1) the hazard classification of the agent; (2) the need for protection of research material or personnel; and (3) the extent to which hazardous aerosols are involved. Review the CDC publication Primary Containment for Biohazards Selection, Installation and Use of Biological Safety Cabinets and contact the EHS Biosafety Officer with questions.

A common mistake among investigators is selecting a Laminar Flow Clean Bench instead of a Class II Biosafety Cabinet.

2. Location and Installation

Because the delicate air curtain created at the front of the cabinet can be easily disrupted, certain considerations must be made to ensure maximum effectiveness of this primary barrier. Consider the following:

- ❑ The BSC should be located away from air supply registers, entrances, windows that open, high traffic areas, and laboratory equipment, e.g. centrifuges, that create turbulence
- ❑ Gas lines should not be installed on BSC's at NC State and the use of gas flame burners in BSC's should be prohibited.

Dimensions of the BSC:

- ❑ Will the BSC need to fit through the door?
- ❑ Will the BSC location fit the ceiling height? (may need 12-14 inches above the BSC for annual certification, lights may need to be moved; if the BSC will be hard-ducted, is there space for duct? etc.)

3. Certification

Before running any performance tests, the BSC shall be properly installed and leveled and airflows adjusted to the nominal set point (+/- 3.0 ft/min [+/- 0.015 m/s]).

Biosafety Cabinet operation, as specified by NSF/ANSI 49 Annex F plus Addendum #1, needs to be verified at the time of installation and annually thereafter. Accredited field certifiers are used to test and certify BSCs. When identifying companies qualified to conduct the necessary field performance tests, contact the EHS Biosafety Officer.

4. Decontamination

Gaseous decontamination is mandatory when moving or surplusing a BSC. It is also required when maintenance work, filter changes, and performance tests require access

to any interior portion of the cabinet. When identifying companies qualified to conduct the decontamination procedure, consult the EHS Biosafety Officer.

Checklist for decontamination prior to moving or surplusing a biosafety cabinet:

- ❑ Contact EHS for a radiation survey, if necessary
- ❑ Disinfect and remove all items from the BSC
- ❑ Surface disinfect the interior of the BSC w/ appropriate disinfectant
- ❑ Remove any Rad. & carcinogen stickers as appropriate
- ❑ Schedule a full gas decon (typically conducted by your certification professionals)
- ❑ Contact building liaison to ensure no HVAC disruption is scheduled at that time
- ❑ Schedule for lab to be vacant during the gas decon procedure
- ❑ Post "DO NOT ENTER" signs at entryways to the decontamination area
- ❑ After gas decon, remove/cover biohazard stickers and
- ❑ Ensure the gas decon vendor posts a label/sign indicating the following:
    1) Vendor contact information
    2) Date and time the gas decon was performed
    3) Method used for gas decontamination
    4) If the gas decon method was successful or not
- ❑ Contact building liaison to disconnect gas lines, vacuum, etc. from BSC
- ❑ If exhaust is hard-ducted, the duct will need to be disconnected

Safe and Effective Use of the BSC

1. Before beginning work:
    a. Monitor alarms, pressure gauges, or flow indicators for any changes.
    b. Shut off the UV light.
    c. Turn the cabinet on and let it run for 3-5 minutes.
    d. Wipe work surface with an appropriate disinfectant, e.g. 70% ethanol.
    e. Place a pan filled with disinfectant or lined with a small biohazard bag inside the BSC to collect discards. Avoid reaching outside of the BSC during procedures to discard waste in floor containers.
    f. Plan your work and place everything needed for the procedure, including the pan for your discards, inside the BSC. Wipe items with disinfectant before placing in BSC.

2. Avoid airflow disruption that could affect the level of protection provided by the BSC:
    a. Keep the BSC free of clutter, e.g. extra equipment and supplies
    b. Don't place objects over the front air intake grille.
    c. Don't block the rear air intake grille.
    d. Limit traffic in the area when the BSC is in use
    e. Make sure lab door is closed, and avoid opening and closing door if located

near the BSC.
   f. Move arms slowly when removing or introducing items.
   g. Keep all materials at least 4 inches inside the sash.
   h. Place a centrifuge or blender that creates air turbulence in the back 1/3 of the cabinet and stop other work while the equipment is running.
   i. Don't operate a Bunsen burner in the cabinet.

3. While working:
   a. Work as far to the back of the BSC workspace as possible.
   b. Segregate contaminated and clean items. Work from "clean to dirty."
   c. Clean up all spills in the cabinet immediately. Allow cabinet to run for 3-5 minutes before resuming work.

4. After completing work:
   a. Wipe down all items with an appropriate disinfectant before removing. Remove all materials and wipe all interior surfaces with an appropriate disinfectant, e.g. 70% ethanol.
   b. Periodically decontaminated under work grilles.

Aerosol-proof rotors and safety cups for centrifuges

Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes splitting or breaking. Follow the procedures below when centrifuging biohazardous materials:

1. Use aerosol-proof rotors or safety buckets with caps that seal with O-rings.
2. Before use inspect O-rings and safety caps for cracks, chips, and erosion.
3. Use tubes with threaded caps. Avoid overfilling the tube and getting caps/closures wet. Wipe tubes down with disinfectant after filling.
4. Load and unload rotors and buckets inside the BSC
5. Balance buckets, tubes and rotors before centrifuging.
6. Disinfect the centrifuge after use.
7. Place small, low-speed centrifuges in a BSC during use to contain aerosols.

Other safety equipment for aerosol-producing devices

The use of certain devices, e.g. blenders, homogenizers, sonicators (ultrasonic disrupters) can produce aerosols. To reduce exposure to aerosols, these devices should be used in a biosafety cabinet whenever possible.

Safety blenders and the BeadBeater homogenizer (BioSpec Products) are designed to prevent leakage of aerosols. The devices should be used in the BSC to prevent accidental release of aerosols.

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable loops and needles can be used.

Chapter 7: Safe Work Practices and PPE

PPE is used to protect personnel from contact with infectious agents and hazardous materials. Supervisors are responsible for conducing workplace assessments and to select and train employees in the use of PPE e.g. lab coats, gloves, safety glasses, face shields, etc. PPE must not be taken home or worn outside the laboratory in non-laboratory areas. For assistance in selecting PPE, contact the EH&S Center.

Personal Protective Equipment:

Personal protective equipment (PPE) is specialized clothing or equipment worn by a lab worker for protection against a hazard. Street clothes are not PPE. The minimum PPE required for the BSL-2 laboratory is no different from standard laboratory PPE or PPE used at BSL-1: lab coats, gloves, and safety glasses (or goggles).

1. Laboratory garments, e.g. lab coats, scrubs, and gowns, are long-sleeved and used to prevent contamination of the skin and street clothes. If splashes may occur, the garment must be fluid-resistant. If required, lab coats should be provided for visitors, maintenance and service workers.
2. Gloves must be worn when working with biohazards. Temperature resistant gloves must be worn when handling hot material or dry ice. If personnel develop or have latex allergies, then nitrile gloves should be used in the lab with biohazards instead of latex gloves. Gloves should overlap the sleeve of the lab garment. Double-gloving adds further protection and is recommended in some circumstances, e.g. for BSL-3 laboratories, or if a spill or splash may occur.
3. Face protection, e.g. goggles or safety glasses with side shields in combination with masks, or face shields, or other splatter guards are required for anticipated splashes or sprays of infectious material.
4. Respirators may be necessary in some cases, e.g. for BSL-3 laboratories. Personnel who require respiratory protection must be evaluated by the UEOHC and trained in respirator selection and usage. Personnel required to wear tight-fitting respirators must be fit-tested by EH&S.

Sharps Precautions:

Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items.

1. Avoid the use of needles and other sharps whenever possible. Many glass items such as Pasteur pipettes have plastic alternatives that should be used.
2. If the use of sharps is unavoidable, take extra precautions and dispose of them immediately after use in the designated puncture-resistant sharps containers. When the container is 2/3 full, submit a hazardous waste collection request from EH&S for its removal. Never allow the container to overfill.
3. Needles must never be recapped, removed from the syringe, sheared, bent or broken. If a needle must be recapped, use a one-handed method or a mechanical device, e.g. forceps.
4. Use a mechanical device to remove scalpel blades, never use your fingers.

5. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Contact EH&S for help in evaluating or selecting safer medical devices, e.g. safe needles or complete the Safety Feature Evaluation Form and submit to Box #8007.

Safe Work Practices:

Proper work practices protect you and others from exposure to infectious materials, reduce the possibility of cross-contamination, and improve the quality of the work performed.

1. Label all equipment used to store infectious materials with a biohazard warning label.
2. Keep an uncluttered work space
3. Plan work procedures with safety in mind
4. Remove PPE and wash hands when leaving the lab
5. Don't eat, drink, smoke, apply cosmetics, and handle contact lenses in the lab
6. Don't mouth pipette
7. Decontaminate work surfaces at the end of an experiment and after a spill occurs
8. Decontaminate reusable PPE as soon as possible after it has been contaminated. Lab coats can be spot treated with 10% bleach or autoclaved before laundering. Never take lab coats home.
9. Protect house vacuum lines and vacuum pumps by using a hydrophobic HEPA filter installed between the collection flask and vacuum source
10. Change gloves often and as soon as possible when visibly contaminated
11. Minimize aerosol production by working carefully
12. Perform procedures that may result in aerosols or splashes in a BSC
13. Use aerosol-proof rotors or safety cups when centrifuging and load and unload them in a BSC

Door Placard for BSL-2 and BSL-3

The laboratory entryway signs are generated by EH&S at the initial completion or update of the Safety Plan. Alternatively, entryway sign revisions for BSL-2 can be initiated during completion of the BSL-2 Checklist or the Biological Use Authorization form. BSL-2 sign information contains the biohazard symbol, biosafety level, and office and after-hours contact numbers for the PI and the second in charge of the laboratory in the PIs absence. BSL-1 laboratories do not post biohazard information onthe entryway sign. The BSL-3 door placard may contain additional information and must be obtained from EH&S.

Disinfection

At NC State University liquid biohazard waste is autoclaved with a test indicator and disposed down the sanitary sewer. Chemicals may NOT be directly poured down the drain. If chemicals are to be used to disinfect liquid media, etc., the disinfectant and contact time must be listed on the BUA and approved by the IBC. For suction flasks, make sure the approved chemical disinfectant is in the flask *before* suctioning off the media.

When decontaminating small tubes such as epi tubes, empty them out into a plastic container in a sink, add a 1:10 dilution of household bleach (5.75% sodium hypochlorite) to water or another IBC approved disinfecting solution. After the appropriate contact time has been achieved (this is listed on the BUA), it may then be poured down the drain.

Characteristics of microorganisms affect their resistance to disinfection. To locate information on proprietary disinfectants, search for the product name at http://ppis.ceris.purdue.edu/htbin/ppisprod.com then refer to the EPA registered disinfectants website at http://www.epa.gov/oppad001/chemregindex.htm to review efficacy claims against microbes of interest.

List of disinfectants*

	Ethylene Oxide	Paraform-aldehyde (gas)	Quaternary Ammonium Cmpds.	Phenolic Cmpds.	Chlorine Cmpds.	Iodophor Cmpds.	Alcohol (ethyl or isopropyl)	Formaldehyde	Glutaral-dehyde
USE PARAMETERS									
Conc. of active ingredient	**400-800 mg/liter**	**0.3 g/ft^3**	**0.1-2%**	**0.2-3%**	**0.01-5%**	**0.47%**	**70-85%**	**4-8%**	**2%**
Temp. (°C)	35-60	>23							
Relative humidity (%)	30-60	>60							
Contact time (min.)	**105-240**	**60-180**	**10-30**	**10-30**	**10-30**	**10-30**	**10-30**	**10-30**	**10-600**
EFFECTIVE AGAINST									
Vegetative Bacteria	+	+	+	+	+	+	+	+	+
Bacterial Spores	+	+			±			±	+
Lipo Viruses	+	+	+	+	+	+	+	+	+
Hydrophilic viruses	+	+		±	+	±	±	+	+
Tubercle bacilli	+	+		+	+	+		+	+
HIV	+	+	+	+	+	+	+	+	+
HBV	+	+		±	+	±	±	+	+
APPLICATIONS									
Contaminated liquid discard					**+**			±	
Contaminated glassware	±		+	+	+		+	±	+
Contaminated instruments	±			+				±	+
Equipment total decontamination	±	+							

*These chemical disinfection methods are recognized by the National Institutes of Health, the CDC, or the American Biological Safety Association.

+ denotes very positive response

± denotes a less positive response

blank denotes a negative response or not applicable

Chapter 8: Biohazard Waste Management

The procedures for Biological Waste and Animal Tissue disposal at NC State are consistent with the North Carolina medical waste rules (15A NCAC 13 B .1200) and the applicable sections of the OSHA Bloodborne Pathogens Standard 29 CFR 1910.1030.

All biohazard waste generated in NC State research laboratories must be properly treated prior to its disposal in designated red dumpsters. If treatment of waste is not an option complete an EH&S hazardous waste collection request.

Biohazard waste that requires treatment prior to disposal in designated red dumpsters includes:

- Materials contaminated or potentially contaminated during the manipulation or clean-up of material generated during research and/or teaching activities requiring biosafety level 1, 2, or 3 or animal or plant biosafety level 1, 2, or 3. Refer to your laboratory's Biological Use Authorization to identify these materials in your lab.
- Liquid blood and body fluids.
- Small amounts of human tissue and anatomical remains.
- Materials contaminated with human tissue or tissue cultures (primary and established) because these are handled at BSL-2
- Animal blood, fluids and bedding from animals infected with BSL2 and BSL3 agents.

Disposal practices for research involving whole animals

Appendix Q of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* specifies disposal practices for research involving whole animals where:

- the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals); and/or
- experiments involving viable recombinant DNA-modified microorganisms are tested on whole animals.

Appendix Q-I-B-1. When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

Appendix Q-I-B-2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

Solid biohazard waste collection and handling procedures:

1. Biohazard waste treatment should only be performed by workers trained under the Safety Plan including Biological Use Authorization and Exposure Control Plan for their work environment.
2. Collect BSL-1 and BSL-2 waste in red biohazard containers lined with a clear autoclave bag.
3. Biohazard Labeling. The hard-walled outer waste collection container must bear the biohazard symbol. Autoclave bags must also have the biohazard symbol of the outside of the bag.
4. Remove bags prior to being 2/3 full to allow headspace to seal the bag for transport to the autoclave. Never overfill you biohazard waste.
5. Bags should be opened before autoclaving to insure sterilization.
6. After treatment in the autoclave, allow the bags to cool. Any breakage of bags or leakage of contaminated materials should be reported to the laboratory director or supervisor at once for instructions on procedures for safe cleanup.
7. Reseal the bags with tape and remove from the building. Place in the red bin marked "Autoclaved" located near the rear of the building.
8. BSL-3 solid waste is collected in orange bags and autoclaved before leaving the containment area according to lab-specific SOPs.

Autoclave performance verification

Each load of biohazardous waste processed in an autoclave must meet the operating conditions and be tested:

1. The operator will incorporate with each load a Chemical Integrator Test Pack (CITP), evaluate the performance of the autoclave based on color changed of the CITP; and document the results in a User Log. A sample Autoclave Use Log is available at the EHS website. All bags autoclaved with a failed CITP will be autoclaved again. 3M SteriGage Test Packs #41360 is currently the system accepted for this test.
2. Users should make sure that the autoclave is working properly before re-autoclaving. If the autoclave needs repair a tag "Out of Service" must be placed on the autoclave.
3. Monthly, a biological challenge will be performed with a standard load. The biological challenge needs to be incubated for 48 hours. Test results will be documented – date tested, initial of person doing test; test results.

Liquid biohazard waste for drain disposal

Liquid biohazard waste from a BSL-3 laboratory is autoclaved following lab-specific SOPs prior to disposal. Autoclaves in BSL-3 labs are validated weekly with biological indicators and a log is kept on-site per the North Carolina medical waste rules.

The preferred method for disinfecting rDNA, BSL-1 and BSL-2 liquid waste for drain disposal is autoclaving on the liquid cycle. If the liquid waste was used for propagating microbes, viral vectors, or toxins, chemical disinfection followed by drain disposal must be listed on your Biological Use Authorization for IBC approval.

Sharps waste collection and handling procedures:

Biohazard sharps waste at NC State is material used with rDNA, BSL-1, BSL-2, or BSL-3 material that have sharp edges capable of causing punctures or cuts, including, but not limited to the following: needles, syringes, scalpels, razor blades, slides, coverslips, Pasteur pipettes, capillary tubes, and broken glass and plastic. Plastic serological pipettes are considered "sharps waste" if they are broken and have a sharp edge.

1. NC State labs collect biohazard sharps waste in labeled plastic sharps containers. The Wake County Landfill will not accept plastic sharps containers from NC State. To avoid injury, do NOT clip, bend, shear, or separate needles from syringes and do NOT recap needles.
2. When the container is ¾ full, cap it, autoclave as applicable, and complete an EH&S hazardous waste collection request. Do not overfill the biosharps container.

Mixed waste:

Mixed waste often requires special procedures. Please contact the EH&S Office for proper disposal procedures.

1. Mixed biological/chemical waste can be disinfected by using carefully selected chemical treatments only if compatible with the other chemicals in the experiment. Handle resulting waste as hazardous chemical liquid waste. Contact the EH&S office for advice on avoiding adverse chemical reactions.
2. Treat animal or human tissue in 10% formalin waste as liquid chemical waste and label the hazardous waste tag "10% formalin + non-infectious animal tissue" or "10% formalin + non-infectious human tissue."
3. Disinfect biologically contaminated radiological solid waste by soaking in a suitable disinfectant. Discard disinfectant waste in designated and posted sink if radiological contamination is within sink disposal limits.
4. Disinfect iodinated liquid waste with a phenolic disinfectant; e.g., Lysol™. Disinfect all other liquid waste with bleach (10% final concentration.) If the waste is within radiological sink disposal limits, dispose of in designated and posted sink. If levels are above sink disposal limits, then package for hazardous waste collection and submit an online request for radiation/chemical waste pick-up.

Chapter 9: Emergencies and Reporting

The North Carolina State University Emergency Information website provides a clearly defined protocol and corresponding support mechanism to protect NC State personnel and property in emergency situations.

The scope of this section is to define emergency situations and specific response procedures to handle injuries, emergencies, and spills that occur in a safe, orderly and efficient manner when research involves biological materials.

Each laboratory space at NC State has a Safety Plan designating a Principal Investigator as supervisor for the space. The Safety Plan contains procedures for spills, contact numbers, and the location of emergency equipment. The PI or designee must review the guide with new personnel and on an annual basis by completing the Supervisor Safety Self Assessment Checklist.

Post the following pages in your lab and/or near biological use areas such as biosafety cabinets:

Injury, Medical Emergency, Animal Bite

OBTAINING MEDICAL ATTENTION

- For serious medical emergencies dial 911.
- For medical treatment during or after work hours, refer to the list of approved local urgent care clinics and hospitals located at http://www.ncsu.edu/ehs/accidents/Clinics.pdf .

HAZARDOUS MATERIAL ON SKIN OR SPLASHED IN EYE

- Remove contaminated clothing, shoes, jewelry, etc.
- Immediately flood exposed areas with water from safety shower, eyewash, or faucet for at least 15 minutes (use soap on skin for biological/blood exposure). Hold eyes open to ensure effective rinsing behind both eyelids.
- Immediately after rinsing, obtain medical attention.
- Review MSDS(s) for hazards and report the incident.

NEEDLESTICK OR CUT WITH CONTAMINATED SHARP ITEM

- Immediately wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, obtain medical attention.
- Report the incident (see below).

INJURY INVOLVING RESEARCH ANIMAL

- BITE/SCRATCH/CUT: wash the area with soap and water for at least 15 minutes.
- Obtain medical attention and report incident to the animal facility.

ASSISTING IN MEDICAL EMERGENCY OR PERSONAL INJURY

- See above OBTAINING MEDICAL ATTENTION.
- Do not move injured person unless there is a danger of further harm from remaining in the location. If the area is unsafe, then evacuate, close doors to area, and prevent access. Provide information to emergency responders.
- Remain with the injured person until medical assistance arrives. Initiate life-saving measures if necessary and you are trained.

Spill procedures for biohazardous material

SPILL INSIDE BIOSAFETY CABINET:

1. Contain spill with absorbent paper.
2. Dampen Paper with disinfectant. Allow to stand for 20 minutes.
3. If sharps/glass are present, use mechanical means to collect the waste (eg. forceps, cardboard flaps).
4. Remove gloves after area is decontaminated.
5. Wash hands.

LARGE SPILL INSIDE BIOSAFETY CABINET:

1. If splash has occurred outside the cabinet resulting in personnel exposure to infectious material, the Principal Investigator and EH&S should be notified and the need for prophylactic treatment or other medical attention determined.
2. Contaminated clothing should be removed and containerized for autoclaving.
3. Thoroughly wash hands and face, if exposure has occurred.
4. Remove gloves after area is decontaminated
5. Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.
6. Spray or wipe walls, work surfaces, and equipment with appropriate disinfectant.
7. Flood top tray, drain pans, and catch basin below work surfaces with disinfectant and allow to stand 20 minutes.
8. Dump excess disinfectant from tray and drain pans into cabinet base.
9. Lift out tray and removable exhaust grille work. Wipe off top and bottom (underside) surfaces with disinfectant sponge or cloth. Replace in position.
10. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.
11. Drain disinfectant from cabinet base into an appropriate container and autoclave.
12. Remove gloves and wash hands.
13. This procedure does not decontaminate the interior parts of the cabinet such as the filters, blowers, and air ducts. If the entire cabinet is to be decontaminated with formaldehyde gas, contact EH&S (515-6858).

SPILL OUTSIDE BSC:

- Decontaminate and/or remove all personnel, clothing and exit laboratory

- Wash hands and any exposed skin thoroughly.
- Alert others in the area. Notify PI and/or EH&S if assistance is required.
- If necessary, allow aerosols to settle for 30 minutes.
- Re-enter wearing PPE (gloves, lab coat, and eye/face protection).
- Cover spill with paper towels and carefully pour disinfectant, e.g., 10% bleach, around and over the spill from outside edges.
- Allow contact time for disinfectant (e.g. 10% bleach for 20 mins).
- Clean-up with paper towels. Pick up sharp items, e.g., broken glass or needles, with forceps or dust pan and brush and place in a sharps container.
- Decontaminate or dispose of clean-up materials in biohazard bag.
- Remove contaminated PPE and wash hands.

BIOSAFETY LEVEL 3 (BSL3) SPILL

- Follow your laboratory-specific SOP for BSL3 biological spills.

Reporting Instructions

Report all injuries, accidents, animal bites, and exposures to your supervisor and follow the Accident Report Form Flowchart to determine which form(s) apply. Forms to be completed are located on the EH&S website at http://www.ncsu.edu/ehs/accidents/accinv1.htm#report .

Chapter 10: Shipping Biological Materials

Training

Most biological materials require specific packaging, labeling, and documentation. Infectious materials (materials containing or expected to contain pathogens affecting humans) are regulated by the US Department of Transportation (DOT) and the International Air Transport Association (IATA). You must complete a hazardous materials shipping training course to be certified to ship infectious biological materials. This training is also required to be able to properly identify your materials according to DOT and IATA guidelines.

EH&S biological material shipping training:
http://www.safety.ncsu.edu/bio_ship_cert/page1.htm

Import and Transfer Permits

Some biological materials require a permit to be imported or transferred to another institution outside of NC State. The importation or interstate transfer of an etiological agent and hosts or vectors of human disease require an import permit from the Center for Disease Control (CDC). This permit applies to the etiological agents themselves, unsterilized biological material (ex: patient samples) containing an etiological agent, and animals that could be a host or vector of disease in humans.

CDC Etiological Agent Import and Interstate Transfers: http://www.cdc.gov/od/eaipp/
The United States Department of Agriculture (USDA) requires a permit for import or interstate transfer of infectious materials affecting livestock and biological materials containing animal material. Tissue culture materials and suspensions of cell culture grown viruses or other etiological agents containing growth stimulants of bovine or other livestock origins are controlled by the USDA due to the potential risk of introducing exotic animal diseases into the US.

USDA Animal and Animal Product Imports and Interstate Transfers:
http://www.aphis.usda.gov/import_export/animals/animal_import/animal_imports.shtml

USDA Plant material Imports and Interstate Transfers:
http://www.aphis.usda.gov/import_export/plants/plant_imports/index.shtml

The U.S. Fish and Wildlife Service requires an import permit for certain live animals.
US Fish and Wildlife Services Permits:
http://www.fws.gov/permits/ImportExport/ImportExport.html

Food (excluding most meat and poultry), drugs, biologics, cosmetics, medical devices, and electronic products that emit radiation, may be subject to examination by the Food and Drug Administration (FDA) when they are being imported or offered for import into the United States. These items must meet the same standards as items available in the US.

FDA import requirements: http://www.fda.gov/ora/import/

Once the permit is granted you will receive the permit and a set of labels which must accompany the shipment upon its arrival in the US. You will have to send these labels to the senders of your materials.

If you are sending a material that requires an import or transfer permit it is your responsibility to ensure the recipient has the proper permits to receive the material before shipping the materials.

Export Licenses

Some pathogens, toxins, and genetically modified organisms require government licenses in order to be legally exported. The Department of Commerce and Department of State regulate the export of some biological materials, chemicals, and equipment. Do not assume that you will not need an export license based on the item's availability in the US. Failure to obtain an export license when one is needed can result in significant fines, loss of export privileges, or jail time.

If you are not certain that the item you are shipping does not need an export license review the Export Controls information found on the SPARCS web page at http://www.ncsu.edu/sparcs/export/index.html . Filing for export control license applications can take several weeks so identify any possible licenses you will need well in advance of your planned shipping date.

Select Agent Transfers

All movements of Select Agents need to be approved and documented even if it is within the University. Contact EH&S if you are considering bringing in a Select Agent, shipping one outside of the University, or moving one from one location on campus to another.

Chapter 11: Biosafety References

Guidelines for Research Involving Recombinant DNA Molecules, National Institutes of Health, April 2002

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, Centers for Disease Control and Prevention, National Institutes of Health, February 2007

Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, BMBL Appendix A, Centers for Disease Control and Prevention, National Institutes of Health

Bloodborne Pathogens Standard CFR 1910.1030, Occupational Safety and Health Administration, U.S. Department of Labor

NC Medical Waste Management Rules, North Carolina Division of Waste Management

North Carolina Biological Agents Registry, North Carolina Department of Health and Human Services

Select Agents Regulations, Animal and Plant Health Inspection Service (APHIS) and the Centers for Disease Control and Prevention (CDC)

Select Agent and Toxin List

A Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents, MMWR Dec 6, 2002/51 (RR-19) 1-8