Laboratory Biosafety Manual

North Carolina State University

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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 in teaching, research, and diagnostic laboratories. 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" at NC State, 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, diagnostic lab directors, and course instructors 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 (see PRR 04.20.09):

    - recombinant or synthetic nucleic acid molecules (Refer to the NCSU Recombinant and Synthetic Nucleic Acid Molecules classification guide) including their use in animals (including arthropods) and plants,
    - human and other primate-derived substances (blood, body fluids, cell lines or tissues),
    - organisms or viruses infectious to humans, animals or plants (e.g. parasites, viruses, bacteria, fungi, prions, rickettsia) or biological materials that may contain these microorganisms;
    - Select Agents or Toxins (human, animal, or plant) – refer to the list here ;
    - biologically active agents (e.g., toxins, venoms) that may cause disease in humans or cause significant impact if released to the environment.

2. Investigators and course instructors 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).

Even if IBC approval is required as stated above, there may still be permits required through NCDACS, USDA, CDC, etc. to import and/or work with materials of biological origin. Principal Investigators are responsible for obtaining all necessary permits regardless of the need to register with the IBC.

Investigators, diagnostic lab directors, and course instructors register projects with the IBC by completing the Biological Use Authorization (BUA) form indicated below. It is recommended that the approved BUA be stored together in a Lab Safety Binder with this manual, the Safety Plan, and other pertinent safety documents such as safety orientation, informed consent, and training for each worker.

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. BUAs are required to assure that an appraisal has been made of the potential impacts associated with the intended use of an organism. All new projects and renewals must be completed using the latest version of the form. The BSO will forward your form to the Institutional Biosafety Committee (IBC) for 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 BUA must be must be renewed every 3 years. Laboratories, courses, or projects with work practices alternative to this Biosafety Manual must include SOPs for such practices with their BUA. Amendments are required to be submitted in writing when the change may have safety consequences but the basic thrust of the study stays the same. In general amendments are appropriate when the change will not involve a change in containment. Contact the Biosafety Officer to discuss your amendment.

The Exposure Control Plan for Bloodborne Pathogens

Any research, diagnostic, or teaching activity conducted 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 also to be 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 as stated above.

The BSL-2 Checklist

In 2007 the CDC enhanced requirements for all research, diagnostic laboratory, and teaching activities conducted at Biosafety Level 2 (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.

Principal Investigator (PI) Responsibilities

For the purposes of this manual, the PI is defined as the faculty member or other person acting in their official capacity as a University representative in whose assigned space a research, diagnostic laboratory, or teaching activity is conducted. The PI is responsible for full compliance with the policies, practices and procedures set forth by NC State. This responsibility extends to all aspects of biosafety involving all individuals who enter or work in the PI's laboratory or collaborate in carrying out the PI's activities. Although the PI may choose to delegate aspects of the Biosafety Program in his/her laboratory to other laboratory personnel (laboratory directors or supervisors) or faculty, this does not absolve the PI of his/her ultimate responsibility. The PI remains accountable for all activities occurring in his/her laboratory. Documentation of training and compliance with appropriate biosafety practices and procedures is essential. The PI is responsible for assuring the appropriate safety training of employees and for correcting errors and unsafe working conditions.

As part of general responsibilities the PI shall:

1. Develop and implement written laboratory-specific biosafety procedures that are consistent with the nature of current and planned research activities and make available copies of the specific biosafety procedures in each laboratory facility. The PI shall ensure that all laboratory personnel, including other faculty members, understand and comply with these laboratory-specific biosafety procedures.
2. Delay initiation of the project until the research protocol has been approved by the IBC.
3. Ensure that all laboratory personnel, maintenance personnel and visitors who may be exposed to any biohazardous agents are informed in advance of their potential risk and of the behavior required to minimize that risk. It is essential that everyone who may have potential exposure to biohazardous agents be informed of such hazards and appropriate safety practices before entering or working with such hazards.
4. Ensure that all maintenance work in, on or around contaminated equipment is conducted only after that equipment is properly decontaminated by the laboratory staff or PI.
5. Ensure that research materials are properly decontaminated before disposal and that all employees are familiar with the appropriate methods of waste disposal. For standard decontamination procedures at NC State, refer to Chapter 8 Biohazard Waste Management of this manual. For specific questions contact the Biosafety Officer (BSO) at 515-6858.
6. Report any significant problems, violations of the policies, practices and procedures to the BSO as soon as reasonably possible.
7. Notify the BSO immediately if:

   a. A laboratory-acquired infection is known or suspected, or
   b. A spill of any quantity involving an agent infectious to humans, plants, or animals occurs in a public area.
8. Receive training in standard microbiological techniques.
9. Ensure that all research personnel are appropriately trained in biosafety and receive appropriate medical surveillance when needed. The PI should refer to Chapter 4 Training of this manual regarding training requirements and contact the BSO for assistance with specific biosafety training needs.
10. Coordinate with the BSO and develop emergency plans for handling accidental spills and personnel contamination.
11. Create and foster an environment in the laboratory that encourages open discussion of biosafety issues, problems and violations of procedure. The PI will not discipline or take any adverse action against any person for reporting problems or violations to the IBC, BSO, Risk Management, or State or Federal agencies.
12. Comply with shipping requirements for biohazardous agents and select agents. EHS conducts shipping training as required for all lab personnel. The PI should refer to Chapter 10 Shipping of this manual and contact EHS to ensure that all applicable transportation safety regulations have been met prior to shipping microbiological cultures, tissues (human or animal) or body fluids. These materials are often regulated for shipment and must only be shipped by personnel who have been properly trained and authorized by NC State to ship such materials on its behalf.

In submitting proposed work to the IBC, the PI shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the requirements set forth by the *NIH Guidelines* (see Chapter 2 of this manual) and the CDC “Biosafety in Microbiological and Biomedical Laboratories” document (see Chapter 3 Risk Groups and Biosafety Levels) as applicable.
2. Select appropriate microbiological practices and laboratory techniques to be used for the research project, diagnostic lab, or lab course.
3. Complete and submit registrations to the IBC using the most current form(s). Registration forms for the use of infectious agents, recombinant or synthetic nucleic acids, and acute biological toxins can be found at: http://www.ncsu.edu/ncsu/ehs/biosafety.htm
4. Submit any significant changes in a given project to the BSO for review and recommendations.
5. Certify that the protocol has been reviewed for research of a dual use concern.

Prior to initiating research, *the PI shall coordinate with the BSO as necessary to:*

1. Make available to all laboratory staff and involved facilities staff (such as animal care staff) the protocols that describe the potential biohazards and the precautions to be taken.
2. Instruct and train all research personnel in:
   a. Identification of the biohazard(s) present,
   b. Practices and techniques required to ensure safety and reduce potential exposure,
   c. Procedures for dealing with accidents, spills and exposures.
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

4. Ensure that collaborators are made aware in advance of any biohazardous agents sent to them, and comply with all applicable packaging and shipping requirements. These materials are often regulated for shipment and must only be shipped by personnel who have received proper training and are authorized by NC State to ship such materials on its behalf.
5. Maintain a formal inventory of all biological material received and sent. Logs should include the approximate quantity of the materials and where it is stored in the laboratory.

During the conduct of the research the PI shall:

1. Supervise the safety performance of the laboratory staff to ensure that required safety practices are employed.
2. Investigate and report in writing to the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures.
3. Immediately notify the BSO of any laboratory spills, accidents, containment failure or violations of biosafety practice which result in the release of biohazardous agents and/or the exposure of laboratory personnel (or the public) to infectious agents.
4. Correct work errors and conditions that may result in the release of biohazardous agents.
5. Ensure the integrity of all containment systems used in the project, lab, or course.
6. Restrict access as required by the laboratory-specific biosafety practices and procedures, and by the biosafety containment level approved by the IBC.
7. Immediately notify the BSO if a Select Agent (see Section V, M; Appendix A of this Charter) or other high-consequence pathogen (i.e. Risk Group 3 or 4) has been isolated and confirmed from environmental and/or diagnostic specimens.

Failure to Comply

Non-compliance with the standards outlined in this Manual can result in severe repercussions for NC State University workers (e.g. disease, injury, death) and the university as a whole (e.g. loss of funding, litigation, etc.). Noncompliance includes, but is not necessarily limited to:

1. Failure to register biohazardous agents, including non-exempt recombinant or synthetic nucleic acid molecules (refer to NCSU Recombinant and Synthetic Nucleic Acid Molecules Classification Guide) ;
2. Failure to provide updates and/or other required documentation within 60 days of the specified due date;
3. Poor biological safety/biological containment practices as documented through lab inspections, routine or otherwise; or
4. Failure to correct a documented (confirmed) biological safety complaint or concern.

Noncompliance will be reported to the IBC which may result in suspension or termination of all approved registrations. The PI's Department Head, Dean, and/or other applicable administrators will be notified of the noncompliance, while granting agencies or regulatory authorities may be notified as required by their respective reporting standards.

Chapter 2: Recombinant and Synthetic Nucleic Acids

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH 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 recombinant DNA technology. The scope of the NIH Guidelines has expanded in recent years to include advances in synthetic nucleic acid synthesis. The purpose of the NIH Guidelines is to specify institutional oversight for the safe handling and containment of (1) recombinant nucleic acid molecules, (2) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (3) cells, organisms, and viruses containing such molecules including transgenic animals and plants.

At NC State, the Institutional Biosafety Committee (IBC) reviews all research covered under the NIH Guidelines. For more information regarding IBC membership, schedules, and procedures, refer to the EHS webpage for NC State University Safety Committees and click on IBC.

Classification of research involving recombinant or synthetic nucleic acid molecules:

To determine whether your research is subject to IBC review at NC State University, refer to the NC State University guide Classification of Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

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

Chapter 3: Risk Groups and Biosafety Levels

When completing the Biological Use Authorization form and whenever planning a new project using potentially hazardous materials or processes, a thorough risk assessment should precede the selection of precautions. A biosafety risk assessment is conducted to determine the appropriate containment for a proposed experiment or project. At NC State University containment consists of a documented set of practices, PPE, equipment, training, and work space designed to protect laboratory employees or students, maintenance or service workers, the public, agriculture, or the environment. This Chapter is intended to provide a starting point for the biosafety risk assessment.

The Risk Group is a comparative descriptor for a given microbe based on the inherent pathogenic nature to (typically) humans. Identifying the Risk Group is usually the first step in the biosafety risk assessment process. The PI may need to provide supporting documentation for each proposed Risk Group on the Biological Use Authorization form (BUA). The four commonly recognized Risk Groups are listed here:

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

How and where the investigator plans to interact with the microbe play a key role in determining containment level (i.e. "biosafety level"). Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the lab, with little safety enhancement. However, if there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.

The PI will propose a biosafety level on the BUA that the IBC will evaluate at the time of registration. There are four commonly recognized biosafety levels as illustrated in the following tables. The proposed biosafety level should be based on a thorough risk assessment that, at a minimum, includes a review of the following resources:

1. The NIH Guidelines Appendix B provides common biological agents used in research listed by Risk Group.
2. Agent Summary Statements for some infectious agents are provided in the BMBL and indicate the appropriate biosafety level for some infectious agents. Section II of the BMBL describes the process of Biological Risk Assessment.
3. The American Biological Safety Association (ABSA) website provides a searchable database of many biological agents and their assigned biosafety levels by country.
4. The Pathogen Safety Data Sheets are produced by the Public Health Agency of Canada as educational and informational resources for laboratory personnel working with certain infectious substances.

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) in the laboratory (see BMBL, Appendix H – Working with Human, NHP and other Mammalian Cells and Tissues). Also, the OSHA Bloodborne Pathogens (BBP) Standard (29 CFR 1910.1030) applies to all work in the laboratory with human 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).

Refer to the Disinfection section of the Safe Work Practices and PPE chapter in this manual to review disinfection, treatment, and disposal requirements for materials covered under the OSHA Bloodborne Pathogens Standard.

Other cultured cells and tissue

Cultured cells which are known to contain or be contaminated with a biohazardous agent (e.g. bacteria or viral) are generally classified at the same risk group as the agent. Cell lines that are not human or other primate cells and which do not contain known human or zoonotic pathogens are often designated for work at biosafety level 1. These may require permits through NCDACS or USDA (Principal Investigators are responsible for obtaining all necessary permits).

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 BSL-2 containment.

General Laboratory Facility Biosafety Levels

The CDC/NIH publication *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 University practices.

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

BSL	AGENT	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to cause disease in humans	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			

Live Vertebrate Animal Work

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 vertebrate

animals are experimentally or naturally infected with agents that infect humans as well as animal agents that may pose theoretical risks if inoculated into humans.

ABSL	Routes of Transmission	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to cause disease in humans	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

Required Procedures for Work in ABSL2 Animal Facilities:

The researcher is responsible for:

1. Registering work with IBC when applicable and providing applicable sections of the BUA to the animal facility director.
2. Communicating the start date of the study and conveying the IBC approved Biological Use Authorization for the animal work to the animal facility director, as well as the manager of the rooms or procedure areas where the hazardous agent work will occur. (Refer to these links for contact information: Laboratory Animal Facility at CVM; Biological Resources Facility on central campus; or Todd See for CALS farm areas
   - **This communication should occur at least five (5) days prior to initiation of the work.**
3. Initiating the work only after obtaining confirmation that your notification has been received.

4. **Placing the proper signs on the animal room door and cages** prior to the initiation of the study.
5. Removing the signs when the study is complete.
    - **Cage Cards and Door Signs:** As soon as the animals have been dosed with the biohazardous agent, cages must be marked with the biohazard cards and the appropriate sign must be posted on the outside of the animal room door by research staff. This sign will be removed by research staff once the infected animals and biohazardous agents are no longer in the animal room.

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. These guidelines specifically do not cover *Drosophila* spp. unless modified in such a manner that they would be of public health concern. 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 and Plant-Associated Organisms

Since plant research usually (but not always) does not pose a human health hazard, biosafety principles are designed instead to protect the natural and agricultural environment.

Plant research involving noxious weeds, invasive plants, and certain plant pests, plant-associated microbes, and plant diseases is regulated by the North Carolina Dept. of Agriculture & Consumer Services, especially when the import, export, or transfer of these or materials is required. Contact the Division of Plant Industry at http://www.ncagr.gov/plantindustry/plant/disease/movpests.htm for rules and regulations specific to North Carolina. Review the Guide to USDA/APHIS permits for federal regulations.

The NIH Guidelines provide containment levels for genetically engineered plants, genetically engineered plant-associated microbes, and genetically engineered plant-associated macroorganisms (arthropods and nematodes) in Appendix P. 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 and Toxins

Select Agents and Toxins are federally regulated because of their potential for use in biological warfare. Click this link to access the latest Select Agents and Toxins List. Materials known to contain agents or toxins on the list (samples, specimens, etc.) are subject to the Select Agent regulations.

Laboratories providing clinical or diagnostic services for humans, animals, or plants must report the identification of a Federal Select Agent or Toxin within 24 hours to (1) the appropriate federal agency (CDC/USDA) and (2) the Biosafety Officer at EHS. Within 7 calendar days of the identification of a Select Agent or Toxin, the Form 4 Report of the Identification of a Select Agent or Toxin must be submitted. These procedures, if not outlined in the Biological Use Authorization form (see Chapter 1), will be requested by the IBC during review.

Before possessing, using, sending, or receiving agents or toxins on the Federal Select Agents and Toxins List, the Principal Investigator, together with NC State University must register with CDC, and/or USDA. A high-level containment laboratory with security enhancements including individual security background checks is required. Contact the Biosafety Officer at EHS for more information on the application and security risk assessment process.

Federally Exempt Quantities of Toxins

The toxins listed below are exempt from CDC and USDA registration requirements if the maximum allowable exempt quantity per Principal Investigator is not exceeded. PI's must submit a Biological Use Authorization form (BUA) to the IBC, document their compliance with the Toxin Due Diligence Provision on their BUA, provide locked storage,

and document their inventories to ensure the 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.

Training has numerous components that include general safety practices and safety theory which progresses to task specific safety practices and Standard Operating Procedures (SOPs), entry and exit procedures, room and suite specific procedures, use of PPE and equipment, animal handling, incident and accident reporting, etc. These components include training under normal operating conditions, during emergencies, systems failures and in the event of a suspect or known exposure. Training is often conducted in a layered approach to include a review of manuals and SOPs, classroom training, hands-on training with a skilled and knowledgeable mentor that may start with less hazardous organisms, progress to a “watch one, do one” approach, and culminate in demonstration of competency.

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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules) at BSL-1 containment.

The link to the Laboratory Biosafety Training exam (accessible from the very last training slide) is here. After successfully completing the exam, an exam confirmation email will be sent to the email address entered on the exam page. Principal Investigators should

instruct all participants to print and maintain this exam confirmation email in their lab safety binder for future reference.

The BUA now contains the Statement of Informed Consent that is designed to be used by Principal Investigators to communicate hazards to laboratory workers and document completion of the online Laboratory Biosafety Training.

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. For more information on training lab workers in biosafety techniques, review the CDC Guidelines for Laboratory Biosafety Competency.

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

Additional Training for PI's Working with Recombinant and Synthetic Nucleic Acid Molecules

Training on the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is required by the NIH of all Principal Investigators with labs working with recombinant or synthetic nucleic acid 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

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 , Medical Surveillance Program , and the PI's approved Biological Use Authorization form.

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, require use of a respirator, 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. Both the Chemical Fume Hood (CFH) and the BSC provide worker protection by enclosing the hazardous operation. However, the CFH is rarely substituted for the BSC because the CFH does not protect the product from contaminating particles found in the surrounding laboratory. 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 .

Biosafety Cabinet Alarms

Do not work in a biosafety cabinet that is in alarm. If your biosafety cabinet goes into alarm, that is an indication that worker, product, and/or environmental protection are compromised. Post the biosafety cabinet prominently with the words "DO NOT USE" and immediately contact your building liaison or the Biosafety Officer.

Biological Safety Cabinets at NC State

Biological safety cabinets can only protect the worker and the product 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 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 Biosafety Officer for ***required*** room air pressure differential test. A radiation survey may also be 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 decontamination (*must* be conducted by a BSC certification vendor, typically the same professionals conducting annual certification)
- ❑ Contact building liaison to ensure no HVAC disruption is scheduled at that time because the gas decon procedure uses high concentrations of toxic gas
- ❑ Schedule for lab to be vacant during the gas decon procedure
- ❑ Post "DO NOT ENTER" signs at entryways to the gas 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. 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.
   b. Periodically decontaminate 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

At NC State, each individual Principal Investigator is responsible for ensuring that proper safety practices, procedures, and equipment are in place. The PI is considered the responsible "supervisor" from an OSHA point of view, regardless of who they delegate these tasks to. Supervisors are responsible for conducing workplace assessments and to select and train employees in the proper use of PPE e.g. lab coats, gloves, safety glasses, face shields, etc. A workplace assessment may be conducted during protocol review, at lab meetings, or while mentoring novel techniques and practices. This assessment should be documented and, if necessary, practices written up as a safety SOP. Considerations for practices at NC State are described below.

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. PPE must not be taken home or worn outside the laboratory in non-laboratory areas. For assistance in selecting PPE, contact the Biosafety Officer or EHS.

The minimum PPE required for the BSL-2 laboratory is no different from standard laboratory 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. EHS offers more guidance at this lab coat selection and disposal link.
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. More info on proper glove selection is available at the EHS website.
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.

General Biosafety 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

Characteristics of microorganisms affect their resistance to disinfection. The Disinfection Selection FAQ (in revision) and the table below provide a starting point for identifying appropriate chemicals for disinfection depending on the circumstances and type of biohazard. 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.

OSHA requires use of an EPA-registered disinfectant under the Bloodborne Pathogens Standard. Note that ***70% ethanol is not an EPA-registered disinfectant***. It evaporates too quickly to be an effective disinfectant. 70% ethanol can be used as a cleaner, for example, to remove excess bleach or other EPA-registered disinfectants. Alternative disinfectants include clorox, amphyl, lysol, and sporicidin.

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. For example, any greater concentration than a 1:5 dilution (20%) of bleach to the final volume needs review of disposal options with the EHS hazardous waste manager. If chemicals are to be used to disinfect liquid media, etc., the final waste product must adhere to all chemical waste disposal regulations. 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.

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. (^{o}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, diagnostic, 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.
- 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.

Tissue, anatomical remains, sharps containers (see below) require removal by EHS.

Refer to this quick reference chart regarding disposal practices of biohazard waste at NC State University.

Disposal practices for research involving whole animals

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

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

Appendix Q-I-B-1. When an animal covered by Appendix Q containing recombinant or synthetic nucleic acid molecules or a recombinant or synthetic nucleic acid molecule-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 Incident 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 chapter 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. **All spills, releases, or accidents involving materials registered on a Biological Use Authorization, regardless of how minor the event or how remote the location, are reported according to the NC State Accident Report Form Flowchart. For questions, contact the Biological Safety Officer at 515-6858.**

There should not be a stigma attached to reporting: humans make mistakes; research is trial and error. Reporting helps provide a safer environment across the university and promotes a positive "safety culture"!

As standard practice, each laboratory space at NC State has a Safety Plan designating a Principal Investigator as supervisor for the space. The Safety Plan contains general procedures for spills, contact numbers, and the location of emergency equipment. PI's are responsible to review safety-related policies and procedures with new personnel during completion of the Manager's Safety Orientation Checklist and on an annual basis during completion of the Supervisor Safety Self Assessment Checklist.

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

Injury, Medical Emergency, Animal Bite

OBTAIN MEDICAL ATTENTION

- For serious medical emergencies day or night, dial 911.
- Minor injuries -- employees: notify your supervisor.
- Minor injuries – students: notify supervisor and report to Student Health Services.

All incidents involving materials registered on a Biological Use Authorization, regardless of how minor the event or how remote the location, are reported to the Biological Safety Officer at 515-6858.

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, see above OBTAIN MEDICAL ATTENTION..

- Report the incident beginning with the First Report of Injury form and notify the Biosafety Officer at 515-6858.

NEEDLESTICK OR CUT WITH CONTAMINATED SHARP ITEM

- Immediately wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, see above OBTAIN MEDICAL ATTENTION.
- Report the incident beginning with the First Report of Injury form and notify the Biosafety Officer at 515-6858.

INJURY INVOLVING RESEARCH ANIMAL

- BITE/SCRATCH/CUT: wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, see above OBTAIN MEDICAL ATTENTION.
- Report the incident beginning with the First Report of Injury form, notify the animal facility manager and notify the Biosafety Officer at 515-6858.

ASSISTING IN MEDICAL EMERGENCY OR PERSONAL INJURY

- See above OBTAIN 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

The quantity of the biohazardous material spilled is not the sole determining factor in deciding whether or not an event is classified as a spill. Rather, the essential issue is whether the biological agent, the location, and the quantity collectively cause the situation to be beyond the control of the lab worker.

Always notify workers in the immediate area if the spill poses a serious threat to their health and safety. If the spill cannot be handled safely by laboratory employees with available absorbents and disinfectants, notify your supervisor and/or dial 911 to notify campus dispatch for assistance.

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 (515-7915) should be notified immediately 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 toxic gas refer, contact the Biosafety Officer at 515-6858.

SPILL OUTSIDE BSC:

1. Decontaminate and/or remove all personnel, clothing and exit laboratory.
2. Wash hands and any exposed skin thoroughly.
3. Alert others in the area. Notify PI and 911 if assistance is required.
4. If necessary, allow aerosols to settle for 30 minutes.
5. Re-enter wearing PPE (gloves, lab coat, and eye/face protection).
6. Cover spill with paper towels and carefully pour disinfectant, e.g., 10% bleach, around and over the spill from outside edges.
7. Allow contact time for disinfectant (e.g. 10% bleach for 20 mins).
8. 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.
9. Decontaminate or dispose of clean-up materials in biohazard bag.
10. Remove contaminated PPE and wash hands.

BIOSAFETY LEVEL 3 (BSL3) SPILL

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

Reporting Instructions

All spills, releases, or accidents involving materials registered on a Biological Use Authorization, regardless of how minor the event or how remote the location, are reported according to the NC State Accident Report Form Flowchart. For questions, contact the Biological Safety Officer at 515-6858.

Report all other injuries, accidents, animal bites, and exposures according to standard practices as outlined in the Accident Report Form Flowchart beginning with the First Report of Injury form. Forms to be completed are also located on the EH&S website at http://www.ncsu.edu/ehs/accidents/accinv1.htm#report .

Chapter 10: Shipping Biological Materials

Transporting Biological Materials

The US Department of Transportation is vigilantly reviewing companies, carriers, and academic settings for compliance in packaging and shipping hazardous materials. It is the responsibility of individual shippers to properly identify their material and package it accordingly. The latest information on shipping biological materials is available on the EHS website for Hazardous Materials Shipping Program.

If you are transporting the materials yourself in a vehicle, even across campus:

- ❑ Use a University Vehicle
- ❑ Collecting specimens? Don't contaminate the vehicle.
- ❑ Check packaging. Sealed secondary container. Biohazard label on the outside.
- ❑ Depending on volume may need spill clean up kit.

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 University. 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), Etiological Agent Import and Interstate Transfers.

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.

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. For more information, review the Guide to USDA Animal and Plant Health Inspection Service (APHIS) Permits.

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.

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: General Biosafety References

American Biological Safety Association Biosafety Links

Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), National Institutes of Health, March 2013

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

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

Select Agent and Toxin List

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