Preface

The use of animals is essential for any institution dedicated to research, testing, and education in the biomedical sciences. Investigations involving animals yield much of the scientific knowledge that is fundamental to our understanding of disease, and they are essential for developing more effective approaches for diagnosis, treatment and prevention. Although techniques not involving animals are available and continue to be developed, animal models remain essential for biomedical research.

The use of animals in research, testing, and teaching carries with it moral, scientific, and legal obligations for the humane care and treatment of the subjects. The Institutional Animal Care and Use Committee (IACUC) plays a central role in the organization of the animal care and use program, and is essential to the University's continued compliance with the various laws and regulations that ensure that animal welfare is maintained. This training module has been prepared to support and educate members of the University community who use animal based models for teaching or research. The module contains a range of information and resources necessary to ethically employ animals in research and teaching in accordance with University policies and Federal regulations. By participating in this training, each member of the University community will help support the University of Pittsburgh's commitment to the safe and humane care and use of research animals.

CHAPTER 1: REGULATIONS IN ANIMAL RESEARCH

CHAPTER 2: THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

CHAPTER 3: ANIMAL MODELS

CHAPTER 4: THE DIVISION OF LABORATORY ANIMAL RESOURCES

CHAPTER 5: UNIVERSITY OF PITTSBURGH OCCUPATIONAL HEALTH AND SAFETY PROGRAM

CHAPTER 6: ANIMAL HAZARDS AND PHYSICAL HAZARDS

CHAPTER 7: BIOLOGICAL AGENTS

CHAPTER 8: CONTROLLED SUBSTANCES

CHAPTER 9: CRITICAL POINTS FOR PERFORMING ANIMAL RESEARCH
Regulations in Animal Research

1. Regulations Mandating Animal Welfare Practice

1.1. The Animal Welfare Act

Public Law 89-544 (the Animal Welfare Act) was originally enacted on August 24, 1966, and has since been amended by Congress on many occasions. The Animal Welfare Act is implemented and regulated by The United States Department of Agriculture (USDA).

Details Regarding the Animal Welfare Act:

• Covers most warm-blooded animals. Birds, rats of the genus Rattus, and mice of the genus Mus are excluded.
• Requires that the institution register with the USDA every 3 years
• Involves regular unannounced inspections by USDA of the registered institution’s facilities and practices.

Provisions are defined more thoroughly by a series of policies enacted by the USDA. These policies are available at: https://www.aphis.usda.gov/animal_welfare/downloads/Animal Care Policy Manual.pdf

The most important USDA policies include:
• Policy 3, Veterinary Care of Animals
• Policy 11, Painful/Distressful Procedures
• Policy 12, Written Narrative for Alternatives to Painful Procedures
• Policy 14, Multiple Survival Surgery

1.2. The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals

The Health Research Extension Act of 1985, Public Law 99-158, entitled “Animals In Research,” was passed by the U.S. Congress on November 20, 1985. This law provides the statutory mandate for the PHS Policy on the humane care and use of laboratory animals, which is available at: http://grants.nih.gov/grants/olaw/references/phspol.htm

This “PHS Policy” allows the Secretary of the U. S. Department of Health and Human Services, acting through the Director of the National Institutes of Health (NIH), to establish guidelines for the following:

• The proper care of animals to be used in biomedical and behavioral research;
• The proper treatment of animals while being used in such research;
• The organization and operation of animal care committees.

The Office of Laboratory Animal Welfare (OLAW; http://grants.nih.gov/grants/olaw/olaw.htm) at the National Institutes of Health has responsibility for the general administration and coordination of the Policy on behalf of the PHS.

The main tenets of the PHS Policy on the Humane Care and Use of Laboratory animals are as follows:

• Governs ALL animal use at institutions that receive funding from the Public Health Service, including research not directly funded by the Federal Government;

• Covers ANY live vertebrate animals used or intended for use in research, research training, experimentation, biological testing, or related purposes;

• Requires a written assurance to NIH that the institution will comply with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The assurance must include information on the institutional program for animal care and use, record keeping, and reporting. Assurances must be renewed at 4 year intervals;

• Depends on the IACUC for enforcement.

1.3. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

These Principles were promulgated in 1985 by the Interagency Research Animal Committee and were adopted by U.S. Government agencies that either develop requirements for, or sponsor procedures involving, the use of vertebrate animals. The Principles were incorporated into the 1986 PHS Policy and provide a framework for research conducted in accordance with the Policy. The following Principles must be adhered to at all institutions that receive funding from the U.S. Federal Government for the use of animals in research:

I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.¹

II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

III. The animals selected for a procedure should be of an appropriate species and quality, and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered as alternatives.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should assume that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain, or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized
animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain, or distress that cannot be relieved, should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for in-service training, including the proper and humane care and use of laboratory animals.

IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned, but should be made, with due regard to Principle II, by an appropriate review group, such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

1.4. American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals

Adherence to the AVMA Guidelines for the Euthanasia of Animals (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf) is required by NIH’s Office of Laboratory Animal Welfare as well as the USDA. These Guidelines were first issued in 1963, and have subsequently been revised seven times, with the last revision in 2013. These Guidelines stipulate acceptable, conditionally acceptable, and unacceptable methods for euthanizing animals.
1.5. Comparing the Animal Welfare Act and NIH Policy

<table>
<thead>
<tr>
<th>Species Covered</th>
<th>USDA Regulations/Animal Welfare Act</th>
<th>NIH Policy</th>
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<tr>
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<td>Warm-blooded animals except mice, rats, &amp; birds</td>
<td>All live vertebrate animals</td>
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<td>Oversight</td>
<td>Unannounced inspections by USDA</td>
<td>Self-monitoring and reporting by IACUC</td>
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<tr>
<td>Sanctions</td>
<td>Fines, revocation of USDA registration, imprisonment</td>
<td>Restriction or withdrawal of grant funding</td>
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<tr>
<td>Implementing Documents</td>
<td>Animal Welfare Act and USDA Policies</td>
<td>NIH Policy on the Humane Care and Use of Laboratory Animals and Guide for the Care and Use of Laboratory Animals</td>
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PLEASE NOTE: Since the University of Pittsburgh receives grant monies from PHS, all users of vertebrate species must comply with NIH policies regarding the use of animals. Users of warm-blooded species other than birds, rats, and mice also must comply with USDA regulations.

2. Requirements of Funding Agencies

Funding Agencies and Foundations

Each funding agency and research foundation has its own set of guidelines regarding the use of animals. Nonetheless, the Guide (Guide for the Care and Use of Laboratory Animals, 2011 edition) is the standard for laboratory animal research, and virtually all funding sources insist on compliance with the Guide. By law, individuals who utilize mammals with the exception of purpose-bred rats and mice in their research and teaching must also comply with the Animal Welfare Act.

AAALAC

- AAALAC is a private, nonprofit body that accredits laboratory animal facilities and animal care programs through peer review, based on the tenets of the Guide and USDA Regulations.

- Every three years a team visits the institution and evaluates both its facilities and policies regarding the use of animals.

- Accreditation by AAALAC is recognized internationally, and is deemed the best means of demonstrating compliance with the Guide and Federal regulations concerning the use of animals.
3. Summary


2. Requirements of Funding Agencies: Each funding agency has unique requirements regarding the use of animals in research. In general, all require compliance with PHS Policies and the Animal Welfare Act. In addition, most funding agencies consider accreditation by AAALAC as being affirmation that an institution maintains high standards with respect to the use of animals.

Every individual who makes use of animals in research or teaching should download and be familiar with the following critical documents:

- Animal Welfare Act and Regulations (if using warm-blooded vertebrates, with the exception of birds, rats of the genus Rattus [laboratory rats], mice of the genus Mus [laboratory mice]): https://www.nal.usda.gov/awic/animal-welfare-act
Institutional Animal Care and Use Committee (IACUC)

1. The Research Conduct and Compliance Office (RCCO)

RCCO Functions:

- Human Research Protection Office (HRPO)
- Institutional Animal Care and Use Committee (IACUC) and IACUC administration
- Radiation Safety Committee and office operations
- Education and Compliance (audit, education, training and certification)
- Institutional Biosafety Committee (IBC) and IBC administration
- Conflict of Interest Committee and office operations
- Human Stem Cell Research Oversight Committee (hSCRO) and office operations
- Office for Investigator-Sponsored Investigational New Drug Applications (IND) and Investigational Device Exemptions (IDE) Support
- Research Integrity
2. IACUC Membership, Office Location, and Organizational Structure

Institutional Animal Care and Use Committee Reporting Structure

George A. Huber, JD MSIE MSSM  
Vice Provost, Research Conduct and Compliance

<table>
<thead>
<tr>
<th>Director</th>
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<tr>
<td>RCCO</td>
<td>IACUC</td>
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<td></td>
<td>Chair</td>
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<td>IACUC Office</td>
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</table>

**Location:**  
Suite 206, Hieber Building  
3500 Fifth Avenue  
Pittsburgh, PA 15213

**IACUC Email:** iacuc@pitt.edu  
**Phone:** 412/383-2008  
**IACUC Web site:** www.iacuc.pitt.edu

Please call or email with any questions regarding the following:

- Writing of IACUC protocols and the review of the documents  
- Training requirements and whether you have completed these requirements  
- Animal welfare regulations

**Animal Care and Use Program Education and Compliance**

**Training Coordinator:** 412/383-1737  
Responsible for documentation and coordination of mandated training programs for all personnel involved in research protocols and animal care

**Compliance Director:** 412/383-2009  
Responsible for auditing components of the animal research program and assuring compliance with existing regulations and policies
IACUC Membership
The IACUC currently consists of more than 35 members representing over 20 different University departments.

Per federal law, the IACUC must include (in addition to scientists):
- a veterinarian
- a non-scientist
- a non-institutional or lay representative

To maintain a committee that represents the broad interests of the University, the Animal Welfare Act states that no more than three individuals serving on the IACUC can come from the same administrative unit.

3. IACUC Duties and Responsibilities
The major function of the IACUC is to establish and maintain measures to ensure the appropriate care and use of all animals involved in research, teaching, and testing. Duties assigned to the IACUC by PHS Policy and the Animal Welfare Act include:

- Semiannually review the University’s Animal Care and Use program to ensure that humane care and use of animals are being maintained.
- Semiannually inspect all animal facilities and animal use areas to ensure conformity with federal guidelines.
- Report to the Institutional Official on the status of the animal research program, and submit an annual report to the NIH regarding the status of the program.
- Review and approve all research and teaching procedures using live animals through protocol review.
- Provide a training program for all personnel involved in using or handling laboratory animals.
- Develop policies that reflect local concerns regarding the use of animals.
4. Protocol Review Process

4.1 Types of Protocol Applications

- **New submissions.** After an initial protocol application is approved, protocols must be re-submitted every three years for as long as the project is active.

- **Renewals.** All IACUC protocols must be renewed annually by completing an abbreviated form.

- **Modifications.**
  - Researchers may request relatively minor changes on an approved protocol (e.g., title, type of euthanasia or anesthesia, <25% increase in the number of animals, etc.) using the modification form.
  - Because any individual coming in contact with animals for research purposes MUST be listed on the relevant protocol, the modification form may be used to add or delete personnel from protocols.

4.2 Protocol Review Process

- Investigators must submit all new IACUC protocol applications via Animal Research Online (ARO), the IACUC's online protocol management system. A Guide for completing online applications is available on the IACUC website. Applications are processed in the order that they are received at the IACUC Office. There are no expedited reviews.

- An IACUC Coordinator responsible for guiding the protocol through the review process, is assigned to each protocol. The Coordinator reviews all applications for accuracy and completeness. Incomplete applications or applications with administrative errors must be returned to the Investigator for revision before being sent to reviewers. The assigned Coordinator also ensures that all required training for the protocol has been completed by each member of personnel listed on the protocol and will notify the Investigator of any pending requirements.

- IACUC protocol applications are assigned to a subcommittee for review. Two primary reviewers from the Subcommittee, along with a veterinarian and hazard specialists, review each application. Applications will also be made available to other IACUC members who may wish to review them.

- After all five Subcommittee reviewers have made comments (within 1-2 weeks), any questions are forwarded to the PI from the IACUC office.

- After the PI's responses and revisions are reviewed by the Subcommittee members and found acceptable, the application is approved.

- A letter approving the protocol is electronically signed by the IACUC Chair and delivered to the PI through ARO. The PI is notified by email that the approval has been granted.

- Any IACUC member may request a full committee review of any protocol. The entire committee meets monthly.

- Any PI may request to address the full committee regarding protocol issues. This request should be made directly to the IACUC Chair (via email to iacuc@pitt.edu).
• **Protocol approval is not an assurance that space is available in the animal facilities. It is recommended that PIs contact the Division of Laboratory Animal Resources (DLAR) for information prior to ordering animals.**

• All animal protocols are reviewed by the Environmental Health and Safety Office to assure that occupational health and safety are maintained. This review process is discussed further in the Occupational Health and Safety section of this training module.

• The Institutional Biosafety Committee (IBC) is responsible for reviewing and approving all work involving synthetic nucleic acids, including recombinant DNA (rDNA), at the University of Pittsburgh. Institutions that perform recombinant DNA research are required by Federal guidelines to have an expert committee in place to review safety procedures for such research. The University of Pittsburgh’s Institutional Biosafety Committee (IBC) is made up of faculty members, as well as two members from the community. Applications to the IBC should be made online at: [https://www.myibc.pitt.edu](https://www.myibc.pitt.edu).

If a study includes rDNA or synthetic nucleic acids, no animals can be ordered, and no experiments can proceed, until approval is gained from the IBC.

• The University’s Human Stem Cell Research Oversight Committee (hSCRO) provides additional oversight for the injection of human stem cells into animals. The hSCRO does not regulate the use of animal stem cells in experimentation. Research that involves 1) the injection of human embryonic stem cells NOT included in NIH’s Stem Cell Registry (see [http://stemcells.nih.gov/](http://stemcells.nih.gov/)) into any animal or 2) the injection of human stem cells into the germ line or brain of any animal must be approved by the hSCRO before the IACUC processes a protocol regarding the research.

The hSCRO application can be downloaded from: [http://www.rcco.pitt.edu/hscro/](http://www.rcco.pitt.edu/hscro/).

Other uses of human stem cells in animal experimentation must be registered with the hSCRO (by completing the hSCRO application). Such registration must be completed before experimentation is initiated, but after the IACUC protocol is approved.

The following offices can provide assistance when preparing specific sections of protocols:

**Environmental Health and Safety:**
Phone: 412-624-9505
Email: biosafe@ehs.pitt.edu

**IBC Office:**
Phone: 412-383-1768
Email: ibo@pitt.edu

**hSCRO Office:**
Phone: 412-383-1766
Email: hscro@pitt.edu
4.2.1 Causes for Delay in Protocol Processing

- Application is incomplete.
- The PI has not responded to questions posed by the IACUC Subcommittee.
- A Subcommittee member has requested a Full Committee review.

4.2.2 Issues that Often Delay Approval

- Training of Project Personnel. All personnel listed as participants on a project MUST have completed the appropriate training before any application can be approved. See section 6 below.
- Rationale for Numbers of Animals Requested. The number of animals requested must be in agreement with the proposed number of groups and the numbers per group. The number of animals per group must be based on statistical power analysis, when possible.
- Experimental Manipulation. Procedures should be described so that reviewers know what will happen to the animal from the time it arrives at the University until euthanized. Experimental manipulations must be detailed clearly and in lay terms. Tables and flow charts are useful in expediting review (PIs should not insert the Methods section of a grant into the application.)
- The experimental endpoint (dependent variable) must be specified.
- Search For Alternatives. The Animal Welfare Act and USDA Policy 12 require that a search for alternatives to potentially painful/distressful procedures, performed on warm-blooded animal species other than rats and mice, be included for protocols employing such procedures. This search must be documented by a description of computer databases considered, as well as inclusive dates and keywords employed in the process. The search can include consultation with experts or attendance at meetings at which alternatives were discussed. Narratives must be provided that are clearly based on the results of the literature search or expert consultation. Although a reference librarian is available for assistance in performing searches (PITT personnel may contact Melissa Ratajeski, Falk Library, 412/648-1971), it is the responsibility of the PI to review the literature uncovered in the search.
- Responses to Reviewers' Concerns. Direct and thorough responses to concerns raised during the review process will greatly reduce protocol turnaround time.

4.2.3 "Red Flag Issues” that require greater scrutiny by the IACUC

- "High profile” studies (i.e., use of nonhuman primates or biohazardous agents)
- Death as an endpoint
- Mouse ascites production
- Multiple survival surgical procedures
- Stress/trauma paradigms, such as cold exposure, etc.
- Prolonged restraint
- Use of hazardous agents
- Food and/or water deprivation
4.2.4 Protocol Numbering Process

The following protocol numbering system is employed by the IACUC Office:

Protocols are assigned an 8-digit number according to the date of approval (first four digits), and a unique place-holder (last four digits):

- Year/month/Place holder ______.
- Modifications are given sequential indicators (-1, -2, -3, etc.) following the protocol number.

Example: Protocol 12020357 was initially approved in February of 2012.

The third modification would appear as 12020357-3.

4.2.5 Protocol Renewal

- A renewal application must be submitted annually, and a new application must be submitted every three years in order to continue work under a given protocol.
- The Principal Investigator will receive a reminder at 90 days, 60 days, and 30 days prior to annual or 3-year protocol expiration dates.
- The Principal Investigator must log in to ARO, call up the protocol, and submit the renewal or new application no less than 30 days before expiration, and respond to all reviewer queries in a timely manner to assure that work can be continued without interruption.
- If a lapse in protocol approval does occur, no further animal ordering will be permitted, and any animals currently on hand will be confiscated. No further experimentation on those animals will be allowed until the protocol has been approved.

4.3 Tips for Securing Protocol Approval

- Prompt response to Committee questions leads to timely protocol approval.
- PIs should submit a completed protocol questionnaire at least one to two months before approval is needed.
- For help in completing their application, PIs should consider a consultation with an IACUC coordinator, faculty IACUC representative, or DLAR veterinary staff member prior to protocol submission.
5. Grant Review

- Grants submitted to the National Institutes of Health and American Heart Association are required to comply with the Public Health Service "Just in Time" policy.
- This policy prohibits the release of grant funding to investigators using vertebrate animals until the IACUC has reviewed the grant application and has ensured that all proposed methods are described in an approved IACUC protocol.
- To comply with this policy, the PI must complete the "Grant Application Review Form," available at: [https://pitt.co1.qualtrics.com/jfe/form/SV_3ZPmDGppaigyld](https://pitt.co1.qualtrics.com/jfe/form/SV_3ZPmDGppaigyld).
- To assure that grant monies are released in a timely manner, the grant review process should be initiated as soon as the PI learns that a fundable score was received.
- The IACUC will review the grant application; if all procedures are appropriately described in the IACUC protocol, the Office of Research will be informed that grant funding can be released.
- For procedures not described in active protocols, the PI will be informed so that new protocols or protocol modifications can be submitted.
- The Office of Research will not release any grant monies to investigators until the IACUC grant review process is complete.

6. Training

- All persons using animals in teaching or research must complete the Use of Laboratory Animals in Research and Education Module (this module).
- In addition, animal users must complete a variety of other training modules, as described on the IACUC training page: [http://www.iacuc.pitt.edu/training](http://www.iacuc.pitt.edu/training).
- Principal Investigators must also ensure that all individuals conducting experimentation on animals are listed on the relevant IACUC protocol. Individuals must be added to the protocol prior to beginning research using animals.
- The issue of pain and/or distress is a critical one. It is the investigator's responsibility to address this issue in depth during the protocol approval process by indicating what procedures will be used to eliminate pain/distress as much as possible during the study.
- To this end, in their book Principles of Humane Experimental Technique (1959), Russell and Birch described the "three Rs" of reduction, refinement, and replacement, to improve animal welfare and "advocate alternatives to live animal use in research." Investigators should be familiar with these principles.

6.1 The 3 R's

- **Reduction**: reducing the number of animals used in a study without jeopardizing statistical validity.
- **Refinement**: decreasing in the incidence or severity of painful/distressful procedures (i.e., the use of genetic models in place of surgically or chemically-induced models).
- **Replacement**: substituting insentient materials (i.e., in vitro methodologies and computer models), for conscious, living, higher animals.
7. **Mistreatment of Animals and Noncompliance**

It is the IACUC's responsibility to investigate all allegations of mistreatment of animals or noncompliance in a thorough and timely manner.

- Good faith reporting of such violations ("whistle blowing") will not be detrimental to an individual's standing within the institution, and discrimination or reprisal for reporting of violations is prohibited by Federal Law. The University of Pittsburgh's Animal Care and Use Committee investigates all concerns regarding the care and treatment of animals. To report a concern, contact one of the following:
  
  o IACUC Chair: 412/623-3233
  o Compliance Director: 412/383-2009
  o Attending Veterinarian: 412/648-8950
  o Any IACUC member or DLAR veterinarian (DLAR on call emergency pager: 412/917-2340)

- Sanctions for noncompliance with University policies regarding animal research may include:
  
  o Letter of reprimand
  o Frequent, unannounced inspections
  o Suspension/termination of the IACUC approval of the respective research study
  o Suspension of further animal orders for the respective study
  o Permanent revocation of animal use privileges at the University of Pittsburgh
  o Notification of funding agency of IACUC actions
Summary

The Research Conduct and Compliance Office (RCCO)
The RCCO Office was formed to bring together most of the regulatory compliance units at the University that oversee biomedical and psychosocial research and teaching. With time, additional regulatory units may join this Office. The responsibility for the Research Conduct and Compliance Office and its constituent regulatory units rests with the Vice Provost for Research Conduct and Compliance.

Organizational Charts and IACUC Staff
In 1985, two government agencies, the United States Department of Agriculture and the National Institutes of Health, implemented regulations that require each research institution that is involved in animal-based research to organize an Institutional Animal Care and Use Committee (IACUC). The two agencies outlined similar requirements for committee functions. The Committee is responsible for the institution-wide animal research program, including review of all proposed research and teaching protocols involving animals. The Committee is responsible to an Institutional Official, who is, in turn, responsible for submitting annual reports on the status of the program to Federal agencies.

Duties and Responsibilities
It is the responsibility of the IACUC to establish and maintain measures that ensure the appropriate care and use of all animals involved in research, teaching, and testing at the University of Pittsburgh.

Protocol Review
A major component of mandated IACUC oversight is the review of all research, teaching and testing protocols involving animals. To accomplish this function the Committee is subdivided into four Subcommittees: three Rodent Subcommittees and one Large Animal subcommittee. The Subcommittees review protocols, and only after all questions and queries made by the Subcommittee have been answered satisfactorily is the protocol given final approval.

Grant Review
Grants submitted to the National Institutes of Health are required to comply with the National Institutes of Health "Just in Time" IACUC review policy.

Training
Federal law mandates that research facilities provide training for personnel participating in animal protocols involving research, teaching and testing.

Principal Investigators must ensure that all individuals conducting experimentation on animals are listed on the relevant IACUC protocol. Individuals must be added to the protocol prior to beginning research using animals. Furthermore, all individuals must be adequately trained in performing procedures on animals before such procedures are attempted.

Whistleblower Policy
It is the IACUC's responsibility and function to investigate all allegations of mistreatment or noncompliance in a thorough and timely manner.
Animal Models

1. Criteria for Choosing an Animal Model
   - Species availability
   - Proper facilities
   - Comparative biology
   - Genetic characteristics
   - Life span
   - Husbandry and technical expertise
   - Space and caging
   - Special environmental or nutritional requirements
   - Biohazard controls
   - Other considerations

2. Advantages and Disadvantages
   There are advantages and disadvantages to using various animal models and the researcher must take this into consideration prior to planning a research project. For additional help in determining the best model for a particular study, a consultation with one of the Division of Laboratory Animal Resources (DLAR) veterinarians is helpful.
   - Advantages of animal models
     - They are predictable and reproducible.
     - Genetic control is easily accomplished.
     - The environment can be standardized.
     - The life span of animals is conducive to experimentation (many species produce multiple generations in a single year).
   - Disadvantages of animal models
     - They are not exact.
     - Extrapolation to man may not always be possible.
     - There may be anatomical and physiological variations.
     - Results may be limited to standardized conditions of the experiment.
     - They generally involve induced states of change, which may differ from the spontaneous condition.
3. Summary

Criteria For Choosing Animal Models

The decision of choosing which laboratory animal species to use in a scientific study can be difficult and should be based on more than precedent. Today, if a scientist is interested in a rodent model, there are hundreds of genetic constructs and mutants from which to choose, as well as the more traditional outbred and inbred stocks. PIs should take their time in making final choices, as they can impact the data generated and the results obtained.

Advantages and Disadvantages

There are advantages and disadvantages to using various animal models, and the researcher must take this into consideration prior to planning a research project. For additional help in determining the best model for study, a consultation with a DLAR veterinarian is helpful.
Division of Laboratory Animal Resources (DLAR)

1. Introduction

The goal of this chapter is to provide an introduction to the Division of Laboratory Animal Resources (DLAR) at the University of Pittsburgh. The DLAR is responsible for supporting animal-based research and teaching through humane animal care and use by providing husbandry, veterinary, surgical, and administration services. The DLAR employs well over 100 dedicated individuals supporting IACUC approved research and teaching at the University of Pittsburgh. The DLAR consists of animal housing, procedure rooms and surgical facilities, equipment, personnel, and programs. The DLAR comprises of 11 separate viviria across a 32 sq. mile geographic area in southwestern Pennsylvania.

2. Mission Statement

- To provide a humane, high quality animal care and use program in compliance with all legal and regulatory requirements including those pertaining to animal welfare, employee occupational health and safety, and research compliance;

- To foster the animal based research enterprise by providing high quality husbandry services and veterinary support;

- To educate both the University’s biomedical research community and the public at large about the value of laboratory animal science;

- To enhance, through high quality animal care and use, the University's national and international reputation for research excellence.

3. Standard Hours of Operation

- Administrative Office: 8:30 AM - 5:00 PM, Monday through Friday.

- Animal Husbandry Services: 7:30 AM – 4:00 PM, Sunday through Saturday. Skeleton crews are assigned on weekends and holidays.

- Veterinary Services: 7:30 AM - 4:00 PM, Monday through Friday or by appointment.

- Surgical Research Services: 7:00 AM – 3:30 PM, Monday through Friday or by appointment.
4. DLAR Management Structure

5. Comprehensive Animal Management System (CAMS)
   - CAMS is a web-based comprehensive software program used by the research community for animal ordering, census management, veterinary records, and billing.
   - CAMS pulls information relevant to DLAR’s animal care operations from IACUC approved protocols contained within the IACUC’s Animal Research Online (ARO) software program, but does not pull the entire protocol.
     - Information shared between ARO and CAMS occurs via daily feeds; keeping information as real-time as possible.
   - Information relating to CAMS is distributed to the research community via the CAMS Listserv. Send an email to cams@pitt.edu to opt in or to opt out of this information service, or go to https://list.pitt.edu/mailman/listinfo/cams_info.
   - CAMS can be accessed via a tab on the DLAR website, www.dlar.pitt.edu, or via https://cams.pitt.edu/. Both, the DLAR website and CAMS are accessible via one’s University HS Connect username and password.
• CAMS tab on DLAR website (https://web.dlar.pitt.edu/CAMS/CAMS_Info.aspx) – the following highlights reflect a host of user tools to acquaint the user with the system, and provide direction on obtaining hands-on training when requested.
  o CAMS ListServe announcements
  o User Training
    ▪ Researcher User Guide
    ▪ Researcher Training Videos
    ▪ For additional hands-on training, contact DLAR Administration at (412) 648-8950
  o Frequently Asked Questions (FAQ)
  o Contacts – personnel supporting various functions and can address user questions specific to the functions within the program.

6. Contact Information
• DLAR Services – a comprehensive list of services, service request forms (fasting, breeding, transfers, research support, etc.), information on contacts, security access, CAMS, etc. is located at www.dlar.pitt.edu. For assistance contact DLAR Administration.
  o Administration (412) 648-8950
    ▪ Email address: dlar@pitt.edu
    ▪ Fax: (412) 648-8449
  o Animal Enrichment: enrich@pitt.edu
  o Import/Export: import@pitt.edu
  o Quarantine: guar@pitt.edu
  o Space Allocation: dlarspac@pitt.edu

• Facility Supervisors are available to assist each investigator. If you do not know the name of your Facility Supervisor, please visit the Contact page on our website, www.dlar.pitt.edu or contact DLAR Administration.

• Emergency Contact Information – contact information is posted within each facility.
  o Husbandry emergency, contact:
    ▪ Facility Supervisor (contact numbers are posted in each facility and on the DLAR website’s Contact page), or
    ▪ Site Manager (contact numbers are posted on the DLAR website’s Contact page), or
    ▪ Associate Director of Animal Care and Operations, pager (412) 958-0247.
Facilities maintenance and animal transport emergency, contact:
- Facility Manager, pager (412) 565-9580, or
- Associate Director of Animal Care and Operations, pager (412) 958-0247.

Veterinary emergency, contact:
- Veterinary Technician on-call by facilities:
  - Site 1: SBST / BST / SRRC / A&S, pager (412) 958-5923
  - Site 2: PBRC / BST3 / RRB, (412) 958-8814
  - Site 3: BTC / MIRM / BRDG2 / HCC, (412) 958-4736
- Veterinarian on-call, pager (412) 917-2340.

Personnel injury emergency, contact:
- Employee Health Services, pager (412) 647-3407 or
- UPMC Presby Emergency Department pager (412) 647-3333.
- The Facility Supervisor.
- If the injury is animal related, also contact the veterinary emergency number.

7. Functions
- Administration
  - Administrative services include:
    - Purchasing (animals, equipment, and supplies)
    - Billing
    - Information Technology
    - Finance
    - Human Resources

- Husbandry – comprised of three (3) primary units
  - Husbandry
  - Facilities Management
  - Transportation

- Animal Husbandry services include:
  - Protocol implementation facility tours and orientation.
  - Facility security access.
  - Space and caging allocation.
  - Twice daily health checks.
  - Daily feeding, watering, and scheduled cage changes.
  - Breeding and weaning.
  - Animal husbandry record keeping and inventory.
  - Consultation on resource allocations for space, equipment and study support.
  - Transportation, quarantine, animal transfer, and export support.
USE OF LABORATORY ANIMALS IN RESEARCH AND EDUCATION

- Scheduled sanitization and maintenance.
- Equipment acquisition, maintenance, and replacement.

- Veterinary Services – comprised of three (3) primary units
  - Veterinary Medical Services
  - Veterinary Surgical Research Services
  - Pathology Services
    - Veterinary Medical Services include:
      - Clinical health care and management of all research animals, including the diagnosis and treatment of spontaneously occurring and research related disease processes.
      - Clinical and research support services through diagnostic laboratories.
      - Quarantine oversight.
      - Animal procurement oversight related to health status, source and regulatory requirements.
      - Occupational Health and Safety program and implementation meetings for hazard studies.
      - Protocol design and budget preparation consultation.
      - Training and advising investigators on animal model selection.
      - Mandated strategy meetings prior to protocol implementation.
      - Institutional Animal Care and Use Committee (IACUC) services, (e.g. protocol review, semi-annual inspections participation, ad hoc subcommittee participation, implementation of IACUC mandated programs, assistance with the development of IACUC training programs.
      - Interact with regulatory agencies and officers.
      - Participation in collaborative research through the application of individual skills and expertise, which include:
        - Anatomic pathology, with special expertise in diagnostic (necropsy and surgical) and infectious disease pathology.
        - Large animal anesthesia, surgery, analgesia and pain management.
        - Rodent colony health management.
        - General nonhuman primate and small animal medicine & surgery.
        - Cardiovascular disease models and pathogenesis.
        - NHP models and infectious diseases.
        - Microbiology and occupational health and safety regulations.
        - NHP colony management, Biosafety Levels 2 and 3 animal experiment design, and rodent disease remediation.
        - General parasitology, and with internationally recognized special expertise in the fields of acanthocephalids and reptilian parasites.
        - NHP socialization, behavior, and psychological enrichment.
        - Rodent import / export logistics, rederivation, cryopreservation, transportation, and external housing arrangements.
o Veterinary Surgical Research Services include:
  - Consultation for the preparation of animal research models, protocol design, and budget preparation.
  - Total anesthesia services (sedation, induction, maintenance, and anesthesia recording).
  - Surgical assistance (first assistant or surgeon).
  - Serum clinical chemistries, hematology, and blood gas analysis.
  - Assistance with post-operative recovery.
  - Assistance with postgraduate surgical training programs (design, protocol, and staffing).
  - Complete sterilizing services (ethylene oxide and steam).

o Veterinary Pathology Services include:
  - Contract research support.
  - General input and advice on subjects such as overall project design, materials, methodologies, developing scoring criteria, etc.
  - Establishing adjunct services such as prosection training, instruction on tissue trimming, digital photomicroscopy, etc.
  - Contract histology processing services and or interpretive services are available via outside vendor.
University of Pittsburgh Occupational Health and Safety Program

1. Overview

The University of Pittsburgh Department of Environmental Health and Safety (EHS) has developed a comprehensive occupational health and safety (OHS) program designed to assess the hazards of animal research protocols, offer guidance on working safely in animal research environments and offer medical education, health screening, vaccinations and injury treatment to workers.

The major elements of the Pitt OHS program, described in this section, include:

- EHS Risk Assessment and Implementation Reviews
- EHS Laboratory Inspection Program
- Medical Surveillance, Screening programs and Injury Treatment

2. EHS Risk Assessment

EHS reviews each IACUC protocol application submitted. Following these reviews, EHS generates a "Risk Assessment" document. This document identifies:

- Biological hazards
- Chemical hazards
- Physical hazards
- Requirements for conducting the project safely
- Medical surveillance requirements and compliance status for all individuals listed on the protocol
- EHS training requirements
- Indication of "Implementation Review" requirements
- Indication of EHS Lab Inspection requirements

The Risk Assessment is generated by EHS and is sent to the PI, IACUC office and DLAR. IACUC protocol approval cannot occur until EHS generates a Risk Assessment.

It is the responsibility of the Principal Investigator to ensure communication of the risk assessment to all individuals listed on the protocol and to ensure adequate training of those individuals to achieve compliance with the recommendations on the Risk Assessment.
3. Implementation Review

During the Risk Assessment process, EHS decides if an "Implementation Review" is required. An Implementation Review is correspondence (it can be done as a meeting, phone conversation or email) between the PI and appropriate campus departments (such as DLAR, EHS or Radiation Safety) before project initiation or animal ordering can occur.

Implementation reviews are necessary to ensure that appropriate controls (training, PPE, signage) are in place to protect workers.

- For example, if an investigator wishes to administer an adenoviral vector (a BSL-2 agent) into mice in the animal facility, the DLAR employees need to be made aware of the project before it begins so that appropriate control measures can be implemented. Therefore the PI needs to contact the DLAR before starting the project so DLAR can review the EHS risk assessment and implement the guidelines for ABSL-2 containment.

If "YES" is indicated on the Risk Assessment for Implementation Review required, the PI must contact the identified departments prior to ordering animals. If a meeting is necessary, it is the responsibility of the PI to coordinate the scheduling of the meeting.

4. EHS Laboratory Inspection Program

EHS conducts a comprehensive inspection program to ensure that work within laboratories is occurring safely. EHS inspects all DLAR facilities and animal housing areas twice yearly for compliance.

EHS also conducts inspections of animal use areas and investigator’s laboratories where work on IACUC protocols occurs. For IACUC protocols, the EHS lab inspection focuses on ensuring:

- All workers are aware of the risks associated with the IACUC protocol, including animal hazards, physical hazards, biological hazards and chemical hazards. The workers should also be aware of measures to mitigate those risks.
- The EHS risk assessment should be readily available for workers to review.
- As applicable, the lab meets the biosafety level (BSL) assigned for the IACUC protocol. This includes verifying facility requirements, engineering controls and work practices.
- As applicable, appropriate chemical hygiene practices are appropriate for the laboratory.

Once the inspection is complete, EHS sends a report to the PI listing observations, opportunities for improvement and critical action items. EHS also sends a copy of the report to the IACUC office and the DLAR.

Depending on the nature of the deficiency, EHS may require follow-up within a specified time frame. EHS may also re-inspect the laboratories to ensure that critical action items have been addressed.
Medical Surveillance and Screening Programs

The University of Pittsburgh has in place a variety of medical surveillance programs specifically for animal users.

The Animal Exposure Surveillance Program (AESP) is intended to provide:

- relevant occupational health and safety information related to use and care of animals;
- clinical evaluation and treatment for individuals with animal related injuries or illnesses.

All University of Pittsburgh faculty and staff are required to participate in this program if they:

- are involved in the care of animals or their living quarters; or
- have contact with animals (live or dead), their viable tissues, body fluids, or waste.

Enrollment in the AESP is achieved by completing a questionnaire regarding medical status, which is utilized to identify health risks during a brief medical consultation with a University appointed clinician at Employee Health Services, Medical Arts Building, 3708 Fifth Avenue, 5th Floor.

Enrollment in the AESP includes:

- an occupational medical history
- safety and health counseling
- appropriate immunizations
- a review of the demands and environmental factors associated with the job, including the type of animal(s) contacted and other potential work-site health hazards
- counseling regarding the bloodborne pathogen exposure control program,
- allergy screening
- an assessment of the level of risk for exposure to pathogens such as tuberculosis, rabies, vaccinia, tetanus, hepatitis B and other zoonotic diseases, including psittacosis, toxoplasmosis and Q Fever

For more information about the AESP, visit http://www.ehs.pitt.edu/AnimalUsers/Animal
Other Medical Screening Programs

In addition to the AESP, a number of agent-specific occupational health programs are in place. Participation in these programs may be required for animal users depending on the type of research being performed. Links to these programs are found below:

More information can be found at www.ehs.pitt.edu by clicking on the "Info for Animal Users" link.

5. Accident Treatment and Reporting

In the event of an animal related injury:

1. Immediately wash and rinse the wound with soap and water. Apply pressure if necessary to control bleeding.
   a. If the injury involves mucous membranes (eye, nose and/or mouth), flush with water for 15 minutes at eyewash station or other potable water source.
   b. If the injury involved a non-human primate (NHP), wash wound immediately for 15 minutes with the E-Z Scrub PCMX brush found in the NHP exposure kit.

2. Report the incident to your immediate supervisor. The supervisor will notify the medical treatment facility indicated below.

3. Proceed to the designated medical treatment facility
   a. In the event of minor injury (i.e. penetrating wound, small laceration, etc.), report to:

   **Employee Health Services**, Medical Arts Building, Suite 500.59, 3708 Fifth Avenue, Pittsburgh PA 15213, phone 412-647-3695

   Clinic Hours are Monday to Friday 7:30 am - 4:00 pm.

   b. During non-Clinic hours, report to:

   **UPMC Presbyterian Hospital Emergency Department**, 200 Lothrop Street, Pittsburgh PA 15213, phone 412-647-3333.

   c. In the event of serious or immediately life-threatening injury (e.g. massive bleeding, loss of consciousness, inability to breathe), report to the

   UPMC Presbyterian Hospital Emergency Department. **Call 811** on the Oakland Campus or **911** off Campus for medical transport.
All injuries should be reported (either directly or via your supervisor) to departmental administrators, so they can be documented on the Employee's Report of Occupational Illness or Injury (worker's compensation form).
Animal Hazards and Physical Hazards

1. Overview

This section will specifically address hazards associated with animals, and will provide information on avoiding, reducing, or eliminating these hazards.

These hazards include:

- Animal allergies
- Animal contact hazards such as scratches and bites
- Sharps injuries
- Ergonomic injuries
- Compressed gases and gas cylinders
- Use of radioactive materials
- Electrical hazards

2. Animal Allergies

Work with animals can create or exaggerate existing allergy symptoms due to exposure to animals and their bedding and cages. The development of an animal allergy is one of the most common occupational health risks associated with animal research.

Allergy symptoms include:

- Sneezing
- Coughing
- Blurry or itchy eyes
- Difficulty breathing
- Skin reactions such as hives

Animal care workers, investigators or staff may experience these symptoms when handling animals or animal cages or bedding, or when working in the animal facility. Other workers in the lab may experience allergy symptoms if animal carcasses are transported out of the animal facility and in to the lab.

Workers can reduce or avoid allergic reactions to animals by implementing:

- **Engineering controls** such as ventilated dump stations for cages and bedding changes, and filter-top cages, limit the discharge of particles into the air.

- **Personal protective equipment** (PPE), such as lab coats and liquid barrier gloves, to avoid exposures of animal allergens to skin or mucous membranes. Each animal facility has garb requirements posted at the entrance and each investigator should establish garb requirements for his/her laboratory. These must be followed at all times.

- **Work practice controls**, such as animals should not be transported out of the animal facility, except in closed containers, and all lab surfaces and equipment in contact with animals and their carcasses should be thoroughly cleaned and
disinfected. Any transport of animals out of the housing facility must be approved by the IACUC.

- An important component of the OHS program is the **Animal Exposure Surveillance Program (AESP)**. The AESP facilitates medical consultation and assessment of animal allergies, for all workers at the University with potential animal contact. The AESP is described in Section 5 of this module.

### 3. Animal Contact Hazards

Work with animals can create or exaggerate existing allergy. Workers can develop infections or illnesses due to naturally occurring pathogens (disease-causing organisms) in animals.
These types of illnesses are called zoonoses or zoonotic diseases. A few prominent zoonoses are listed below:

<table>
<thead>
<tr>
<th>Zoonosis</th>
<th>Animal reservoir</th>
<th>Symptoms</th>
<th>Transmission</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes B virus (Cercopithecine herpesvirus)</td>
<td>Rhesus macaques, pig-tailed macaques, and cynomolgus monkeys.</td>
<td>Fever, rash, weakness, nausea, leading to fatal encephalomyelitis.</td>
<td>Needlestick, animal bite or scratch, splash of blood or body fluids or feces to mucous membranes.</td>
<td>Surveillance and history of animals. Although animals that repeatedly test negative may harbor the virus BSL-4 housing of infectious animals.</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis virus</td>
<td>Wild mice. Reported in mice, rats, guinea pigs, hamsters, non-human primates, swine, dogs.</td>
<td>Flu-like illness, rash, enlargement of lymph nodes.</td>
<td>Inhalation, needlestick, splash to mucous membranes, exposure to aerosols from bedding and feces.</td>
<td>Animal surveillance. Screening of all tumor cells and cell lines.</td>
</tr>
<tr>
<td>Q fever (Coxiella burnetii)</td>
<td>Sheep, goats, cattle. Reported in cats and rabbits.</td>
<td>Flu-like illness with fever, headache, chest pain, with potential for liver or cardiac illness.</td>
<td>Exposure to aerosols from urine, feces, milk, and birth products.</td>
<td>Animal surveillance. Animal biosafety level 3 housing of infectious animals.</td>
</tr>
<tr>
<td>Rabies</td>
<td>Wild dogs, bats, raccoons. Reported in domesticated dogs and cats.</td>
<td>Anxiety, fever, headache, leading to fatal viral infection.</td>
<td>Bite or splash of saliva to mucous membrane, exposure to aerosols in caves with roosting bats.</td>
<td>Pre-exposure immunization of animals and workers.</td>
</tr>
<tr>
<td>Toxoplasmosis (Toxoplasma gondii)</td>
<td>Parasite. Cats and other mammals (sheep, goats) are infected within a few weeks of initial exposure to the parasite.</td>
<td>Flu-like symptoms, occasional eye damage. Eye and brain damage in infants born to mothers exposed to parasite for the first time during or just before pregnancy.</td>
<td>Skin contact or inhalation of particles from contaminated feces.</td>
<td>Surveillance of workers to determine if exposure has ever occurred. Prior exposure usually confers immunity, except in the case of immunocompromised persons. BSL-2 housing of infected animals.</td>
</tr>
<tr>
<td>Tuberculosis (Mycobacterium tuberculosis)</td>
<td>Non-human primates. Reported in mice, cats, swine, and rabbits.</td>
<td>Infection of pulmonary system and potential for systemic dissemination.</td>
<td>Repeated close contact with exposed individuals or animals, especially aerosols produced by coughing.</td>
<td>Surveillance of incoming animals; periodic monitoring of animals; quarantine of infected or suspicious animals; periodic surveillance of workers.</td>
</tr>
</tbody>
</table>

References for Zoonoses:

- Centers for Disease Control and Prevention (CDC): http://www.cdc.gov/onehealth/zoonotic-diseases.html
Workers can reduce or avoid exposure to naturally occurring pathogens in animals by:

- Understanding the diseases and routes of transmission associated in the animal species they handle.
- Following garb requirements and required medical surveillance in the animal facility and in the lab, e.g. TB testing every 6 months is required for entrance into non-human primate areas.
- Asking the supervisor or investigator about the animals that are handled, and if animals have been tested for certain agents.
- Using non-human primate exposure kits located in primate housing and procedure rooms, and contain equipment such as treated scrub brushes to clean a wound site. Exposure kits are available from the DLAR.

4. Physical Injuries

Work with animals can cause physical injuries, such as scratches and bites, and splashes and sprays of urine and feces.

- Animals may try to bite or scratch workers.
- Non-human primates may try to reach through their cages.
- Large animals, such as cow's goats, and horses, may kick.
- Cleaning solutions such as detergents and disinfectants may splash a worker's unprotected skin or eyes.
- A needle stick can result from improper disposal of used needles.

Workers can reduce or avoid physical harm when working with animals by:

- Following proper animal handling techniques as discussed in the required animal training sessions. (Link is http://www.iacuc.pitt.edu/training.)
- Following established procedures for transporting animals.
- Using engineered safety devices such as primate squeeze cages or self-sheathing needles.
- Following the garb requirements for the facility or procedure.
- Ensuring that eyewashes and safety showers are accessible and operational.
- Check eyewashes weekly for proper operation.
5. **Sharp Injuries**

Sharps can include the following:

- Needles
- Scalpels and razors
- Broken glass
- Scissors

Ways to reduce and avoid sharps injuries:

- Do not reuse needles.
- Dispose in approved sharps containers.
  - Do not overfill sharps containers; fill to about two-thirds full, then close the lid.
  - Never dispose of sharps in the regular trash.
- Do not recap needles.
  - Recapping needles with both hands may result in a needle stick.
  - Needles do not need to be recapped; the needle should be disposed in an approved sharps container.
- Use engineered shapes devices when possible.
  - Engineered devices include self-sheathing needles, self-blunting needles, and the needleless delivery systems.
  - The EHS website provides more information on engineered needle safety devices at www.ehs.pitt.edu.

6. **Ergonomic injuries**

Ergonomic injuries can result from handling animals or equipment.

- Cage racks are heavy and must be moved appropriately.
- Bending and lifting improperly can cause back strain.
- Fingers may get pinched if equipment is not moved properly.

Workers can reduce or avoid ergonomic injuries by:

- Following established procedures for moving equipment or lifting animals.
- Using mechanical aids to move heavy objects, such as a hand truck or dolly to move drums of detergents and chemicals, and wheeled carts to move animals in cages.
- If there is no procedure, discuss the process with the supervisor or investigator and make a plan of action for the safe handling of the animals or equipment.
- Report all injuries promptly to your supervisor.
7. **Other Hazards**

**Compressed gases**

- Gas cylinders must be secured with approved straps, chains, or brackets.
- Gas cylinders must be transported using a hand truck or dolly.
- These principles apply whether cylinders are full or empty.

**Radioactive materials**

- Workers must be trained and follow all requirements set forth by the Radiation Safety Office. (Link is [http://www.radsafe.pitt.edu](http://www.radsafe.pitt.edu).)

**Electrical hazards**

- Water sources must be identified and kept away from electrical outlets or electrical equipment. Electrical equipment should not be located at sinks.
- Extension cords must be kept off the floor and out of areas of pedestrian traffic. Extension cords should only be used as a temporary source of power.

**Noise hazards**

- Loud equipment should be relocated to separate areas when possible.
- Barking dogs may create a noise hazard.
- Noise monitoring and noise protection is available from EHS. (Link is [www.ehs.pitt.edu](http://www.ehs.pitt.edu).)
Biological Agents

1. Overview

This section will specifically address hazards associated with the use of biological agents in animals, and will provide information on avoiding, reducing, or eliminating these hazards.

These agents include:

- Bacteria
- Viruses or Viral vectors
- Toxins of biological origin
- Human cells, either primary cells or cell lines, body fluids and tissues
- Non-human primate blood, tissue, and body fluids are automatically considered as biohazardous.

The use of biological agents in animals is a health and safety concern for several reasons.

- Animals may shed these agents for an unknown period of time in urine or feces, contaminating the bedding and cages.
- Animals may develop a systemic infection, making their blood or tissues contagious.
- Workers injecting animals with agents could have an accidental needlestick and become infected or experience an inflammatory response.
- Other workers in the lab may become at risk for exposure to biological agents if animals or animal carcasses are transported out of the animal facility and to the lab.
- The public may be exposed to biological agents during transport of animals if appropriate precautions are not in place.

2. Animal Biosafety Level (ABSL)

To determine the controls that must be in place to safely handle a biological agent, it is necessary to understand the Biosafety level (BSL) and Animal Biosafety Level (ABSL) at which an agent must be handled.

The Biosafety Level (BSL) represents the conditions under which a particular biological agent can be handled safely, including practices, equipment, personal protective apparel, and facilities.

The Animal Biosafety Level (ABSL) represents the conditions under which a particular biological agent can be handled safely in animals, including appropriate animal housing such as caging and facilities, and handling of exposed tissues and bodily fluids.
There are four biosafety levels (BSL-1 through BSL-4) and corresponding animal biosafety levels (ABSL-1 through ABSL-4.)

At the University of Pittsburgh, BSL-2+ may also be designated. A BSL-2+ designation means that research is approved for a BSL-2 facility, but BSL-3 work practices shall be followed at all times.

Biosafety level 4 agents are not handled at the University of Pittsburgh.

### 2.1 Animal Biosafety Level 1 (ABSL-1)

<table>
<thead>
<tr>
<th>Animal biosafety level</th>
<th>ABSL-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent description</td>
<td>Agents that do not cause disease in healthy adults.</td>
</tr>
<tr>
<td>Examples of agents</td>
<td>E. coli endotoxin, Plasmid DNA, &quot;Naked&quot; DNA, Rat and mouse primary cells, cell lines, tumors.</td>
</tr>
<tr>
<td>Practices</td>
<td>Standard animal care. Participation in Animal Exposure Surveillance Program. Label the cage cards with the full name of the agent (no abbreviations.)</td>
</tr>
<tr>
<td>Safety equipment</td>
<td>Normal housing facilities and cages. Established garb requirements for the housing facility.</td>
</tr>
<tr>
<td>Facilities</td>
<td>Standard animal facility. No recirculation of exhaust air.</td>
</tr>
</tbody>
</table>

### 2.2 Animal Biosafety Level 2 (ABSL-2)

<table>
<thead>
<tr>
<th>Animal biosafety level</th>
<th>ABSL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent description</td>
<td>Agents that may cause disease in healthy adults, but that are not spread via airborne transmission. Hazards include needlestick, ingestion, spills or splashes, aerosols.</td>
</tr>
<tr>
<td>Examples of agents</td>
<td>Human cell lines, Human primary cells, Adenovirus and vectors, Adeno-associated virus (AAV) and vectors, Herpes simplex virus and vectors</td>
</tr>
<tr>
<td>Practices</td>
<td>ABSL-1 practices plus DLAR Implementation Review: Meeting with veterinarian and animal facility supervisor. Selection of appropriate housing area and cage type. Identification of biosafety cabinets for administration of biohazardous agents to animals and cage changes. Biohazard warning signs on the housing room door (responsibility of animal facility supervisor.) Biohazard stickers, and the full name of the biological agent, on the cage and cage cards (responsibility of investigator.) Decontamination of all infectious wastes and of animal cages prior to dumping and washing.</td>
</tr>
<tr>
<td>Safety equipment</td>
<td>ABSL-1 equipment plus primary barriers: When possible, microisolator cages for animals. Certified biosafety cabinet for administration of biohazardous agents to animals and cage changes.</td>
</tr>
<tr>
<td>Facilities</td>
<td>ABSL-1 facility plus: Autoclave available to decontaminate biohazardous bedding and cages. Housing rooms available under negative pressure.</td>
</tr>
</tbody>
</table>
### 2.3 Animal Biosafety Level 2 Plus (ABSL-2+)

<table>
<thead>
<tr>
<th>Animal biosafety level</th>
<th>ABSL-2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent description</td>
<td>The use of biosafety level 3 practices in biosafety level 2 facilities.</td>
</tr>
<tr>
<td>Examples of agents</td>
<td>Agents which have specific risk factors as defined by the NIH, but are not airborne transmissible. HIV (Human immunodeficiency virus) and vectors SIV (Simian immunodeficiency virus) and vectors</td>
</tr>
</tbody>
</table>
| Practices              | ABSL-2 practices plus:  
A standard operating procedure must be developed and followed for work at BSL-2+.  
The investigator must prepare an operations manual that describes the safe handling of the agent.  
Access restrictions for individuals not working with agents. |
| Facilities             | ABSL-2 housing. |

### 2.3 Animal Biosafety Level 3 (ABSL-3)

<table>
<thead>
<tr>
<th>Animal biosafety level</th>
<th>ABSL-3</th>
</tr>
</thead>
</table>
| Agent description      | Agents that can cause disease in healthy adults, and can be spread via airborne transmission.  
Medical treatment is available for the agent. |
| Examples of agents     | Tuberculosis (Mycobacterium tuberculosis)  
West Nile virus  
Hantavirus |
| Practices              | ABSL-2 practices plus:  
The investigator must prepare an operations manual specific to the use of the BSL-3 agent.  
All employees must read and follow the procedures outlined in the BSL-3 operations manual.  
Garb requirements specific to the agent in use are established and followed by all employees.  
Liquid and solid wastes must be decontaminated within the BSL-3 facility, preferably via steam sterilization, prior to removal from the facility and disposal. |
| Safety equipment       | ABSL-2 equipment plus:  
Animals exposed to BSL-3 agents must be housed within a BSL-3 animal facility.  
Agent must be handled only in a certified biosafety cabinet.  
Agent must be centrifuged only in a unit with sealed rotor lids or rotor caps. Rotor lids should be opened only in the biosafety cabinet.  
Steam sterilizer (autoclave) must be available for decontamination of liquid and solid wastes. |
| Facilities             | Agent must be handled only in a BSL-3 facility that has been commissioned and inspected by EHS.  
The facility must be signed as biosafety level 3. |

### 3. References for Biological Agents

- University Standard Operating Procedures (SOPs) exist for a number of biological agents, including rabies virus, vaccinia virus, lentivirus such as HIV (Human immunodeficiency virus), measles virus, etc.

- EHS provides **Risk Assessment** for the use of biological and chemical agents described in each approved IACUC protocol. The **Risk Assessment** is further described in Chapter 5 of this module.

- CDC has information on common disease agents. General website is:  
  [http://www.cdc.gov](http://www.cdc.gov)


4. How to Reduce or Avoid Exposure to Biological Agents

4.1 Evaluate experiments to "build in" safety.

Which agents must be used for the purpose of the study? Can less-hazardous agents be substituted? Examples:

- If tumor growth in animals is desired, then inject animals with murine tumor cell lines, instead of injecting them with carcinogens that could be excreted in bedding or human cell lines that could contain a human pathogen.

- If viruses are used, then use replication-defective viruses that replicate only once to produce the desired gene expression, instead of replication-competent viruses.

If viruses are used, then use less-hazardous viruses, such as murine retroviruses, instead of more hazardous viruses such as HIV or vaccinia virus.

4.2 Facility Design

- Animals exposed to BSL-2 agents must be housed in appropriate housing for the agents used. Designated biohazard housing areas must be kept under negative pressure.

- Animals exposed to BSL-3 agents must be housed in appropriate housing for the agents used. Designated BSL-3 facilities must be commissioned and inspected.
  - ABSL-3 areas must be kept under negative pressure with respect to nearby corridors and housing areas.
  - Environmental controls must be designed to prevent any air from leaving the ABSL-3 area unless it has been HEPA-filtered first.
  - Air supply and exhaust systems should provide 100% of outside air, and it should be directly vented outside the building after it passes through a HEPA filter.
  - Redundant systems should be considered for all environmental controls.
4.3. **Use appropriate engineering controls.**

- Handle agents in biosafety cabinets to limit workers' exposure to spills, splashes, or aerosols.
- House animals in microisolator cages to prevent particles or aerosols from contaminating the room air or fomites. Use ventilation racks when possible.
- Change cages in biosafety cabinets or downdraft tables to control exposure to agents excreted in urine or feces.
- Dump bedding in biosafety cabinets or controlled bedding dump stations.
- Dispose of all sharps in approved sharps containers.
- Animals exposed to biohazards must be transported in microisolator cages or in filter-top containers.
- EHS is available to assist with selection of engineering controls.


4.4. **Follow appropriate work practices.**

- Wear appropriate personal protective equipment (PPE)
  - Animal facilities have garb requirements that include protection against biological agents.
  - Wearing lab coats provides basic protection.
  - Wearing disposable lab gowns, when working with hazardous agents such as HIV, also provides basic protection.
- Decontamination of all surfaces where agents or exposed animals are handled must occur.
  - Select an EPA registered disinfectant appropriate to the biological agent and follow contact time recommendations.
  - Clean up all spills or splashes of biological agents.
  - Disinfect all areas in contact with animals or associated equipment.
- Dispose of all solid wastes associated with the agent, such as used test tubes or microcentrifuge vials, in biohazard bags.
Controlled Substances

1. What is a Controlled Substance?

- A substance whose manufacture, importation, possession, use, and distribution is regulated under the Controlled Substances Act (CSA; http://www.fda.gov/regulatoryinformation/legislation/ucm148726.htm), a federal statute.
- The particular substances covered by CSA were originally stipulated by Congress when CSA was passed by the 91st Congress in 1970 (and signed into law by President Nixon).
- Two federal agencies, the Drug Enforcement Administration and the Food and Drug Administration, determine which substances are added to or removed from the list covered by CSA.
- The Drug Enforcement Administration (DEA), part of the Department of Justice, is the lead agency for enforcement of CSA.
- A number of anesthetics and analgesics commonly used for animal research are regulated through the Controlled Substances Act.

2. Classification of Controlled Substances

- All drugs and chemicals regulated under CSA are placed in one of five “schedules,” according to medical utility and potential for abuse.
- For more information, see: www.dea.gov/druginfo/ds.shtml
  - Schedule 1 chemicals include marijuana and its cannabinoids, MDMA (ecstasy), mescaline, peyote, heroin, LSD.
  - Schedule 2 drugs include codeine, morphine, oxycodone, cocaine, pentobarbital.
  - Schedule 3 drugs include ketamine and testosterone.
  - Schedule 4 drugs include xanax, darvocet, valium, ativan, ambien.
  - Schedule 5 drugs include lomotil, motofen, Lyrica, parepectolin
3. Complying with CSA: licensing

- The PI of a lab using controlled substances must hold a DEA registration. Use of controlled substances purchased under another investigator’s DEA registration is not permitted unless:
  - The other investigator’s DEA license specifies the same use and storage location.
  - The other investigator is listed on the animal protocol the study is related to.

- A clinician’s practitioner’s license may be used to acquire controlled substances for the laboratory provided:
  - The address listed on the license corresponds to the address where the drugs are used and stored.
  - For example, if a physician’s laboratory and lab are in Scaife Hall and their clinical practice is in Presbyterian Hospital (considered one site by the Pittsburgh DEA office), then their practitioner license can be used for both lab and clinical work.
  - However, if a physician’s laboratory and lab are in Scaife Hall and their clinical practice is in Children’s Hospital, then two DEA licenses are needed.

Application for a DEA license should be made online at: [http://www.deadiversion.usdoj.gov/drugreg/](http://www.deadiversion.usdoj.gov/drugreg/)

Note that Schedule 1 registrations must be submitted by mail.
4. Complying with CSA: security

- All controlled substances must be double-locked when the registrant or their authorized agent are not present.
  - If the laboratory door is always locked, this can serve as one the locks.
  - Within the laboratory, controlled substances must be secured within a locked cabinet or safe that cannot be moved or transported.
  - Schedule I and II controlled substances must be secured within a specific type of safe or steel cabinet. See: www.deadiversion.usdoj.gov/21cfr/cfr/1301/1301_72.htm

- Small amounts of controlled substances to be used in the near future can be removed from secured locations.
  - Containers (e.g., syringes) containing the materials must be labeled with:
    ✓ Identity of the pharmaceutical
    ✓ Name of DEA licensee
    ✓ Expiration date
    ✓ Date mixed
    ✓ Concentration

- Each DEA registrant must maintain controlled substances in a separate secured location.
5. Complying with CSA: control and accountability

- Only the DEA licensee and “authorized agents” may have access to the locked drug cabinet.
  - Each lab can only have a limited number of authorized agents.
  - Authorized agents must complete a security background screening through the human resources department (HR). See: www.hr.pitt.edu/sites/default/files/dea_hr_procedures_0.pdf

  ✓ The DEA licensee and potential authorized agent must complete the HR form.
  ✓ The potential authorized agent must report to HR with the form, and complete additional paperwork to authorize a background screening.

- HR will provide documentation to the DEA licensee when the background check is complete.
- This documentation should be maintained with other controlled drug paperwork.
- Note that there is no need to notify HR when an authorized agent leaves the lab.
6. Complying with CSA: inventory

- A log of usage of controlled substances must be maintained.
- This log will be reviewed during inspections conducted by the IACUC and Environmental Health and Safety (EH&S).
- This log will be reviewed by the DEA during audits.
- A sample log sheet can be downloaded from:
  [http://www.rcco.pitt.edu/ControlledDrugs/CS%20Accountability%20Record.doc](http://www.rcco.pitt.edu/ControlledDrugs/CS%20Accountability%20Record.doc)

### UNIVERSITY OF PITTSBURGH
CONTROLLED SUBSTANCES ACCOUNTABILITY RECORD

<table>
<thead>
<tr>
<th>Controlled Substance:</th>
<th>Strength (e.g.; mg/ml, mg/tab):</th>
<th>Container Qnt.: ml</th>
<th>tabs/caps</th>
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<tbody>
<tr>
<td>Container ID:</td>
<td>Expiration Date:</td>
<td>DEA Schedule:</td>
<td></td>
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<tr>
<td>Date Received:</td>
<td>Vendor:</td>
<td>Container Integrity (@ receipt): OK</td>
<td>Not OK (Disposition: )</td>
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<tr>
<th>QUANTITY</th>
<th>ml</th>
<th>tabs/caps</th>
<th>DATE</th>
<th>IN</th>
<th>OUT</th>
<th>ON HAND (if applicable)</th>
<th>ANIMAL ID</th>
<th>PROTOCOL #</th>
<th>PURPOSE</th>
<th>DISPENSED BY</th>
<th>DISPENSED TO</th>
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Final Disposition: [ ] Used up on date: ______ | | [ ] Discarded per DEA instructions (______) on date: ______

### INVENTORY RECONCILIATION

<table>
<thead>
<tr>
<th>DATE</th>
<th>ON HAND (RECORD)</th>
<th>ON HAND (ACTUAL)</th>
<th>INITIALS</th>
<th>INITIALS - DEA Registrant</th>
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1. Use one form for each container (e.g., vial, bottle) received.
2. Label each new container individually and in consecutive order. Use oldest container first.
3. Dispensing of controlled substances is restricted to the DEA Registrant or Authorized Agents of the DEA Registrant (see University policies).
4. Receipt of controlled substances is restricted to the DEA Registrant or Authorized Laboratory Personnel of the DEA Registrant (see University policies).
5. Summarize method of discard (e.g., “Reverse distributor”)
6. Signature (initials) of responsible Authorized Agent of DEA Registrant, if applicable
7. Signature (initials) of DEA Registrant

- At least quarterly, the DEA registrant or authorized agent should conduct an audit to reconcile the current inventory of controlled substances.
- At least every two years, an inventory of controlled substances on hand must be performed and documented.
USE OF LABORATORY ANIMALS IN RESEARCH AND EDUCATION

- If there is a theft of controlled substances, the DEA registrant must:
  - Report the loss to the University of Pittsburgh police department immediately
  - Report the loss to the Pittsburgh DEA office within 24 hrs

- If there is a loss of controlled substances (e.g., you drop and break a vial), the DEA registrant must record the loss in the drug inventory.

- Once a vial is empty, it can be discarded.

- If a vial expires before the contents are used:
  - It must be marked as expired and placed in a separate, secured location (e.g., in a box in the drug safe marked as "expired").
  - The contents must not be discarded as waste.
  - The vial should be disposed of via a reverse distributor.

  ✓ EH&S arranges for quarterly, free disposal. For more information, see: [http://www.ehs.pitt.edu/workplace/waste.html](http://www.ehs.pitt.edu/workplace/waste.html)

--7. Ordering Controlled Substances at the University of Pittsburgh

- Under the Pennsylvania Controlled Substance, Drug, Device, and Cosmetic Act, researchers who purchase prescription drugs (including controlled substances) and devices for lab research are exempt from PA state registration or licensure provided the research is within the scope of an approved research protocol.

- A letter of authorization from the PI's Department Chair or Dean is needed to confirm their need to order prescription drugs (*Certification of Exemption*).

- Only one vendor can be used for controlled substances (unless they do not have the drug in stock): Henry Schein Animal Health ([http://www.henryscheinvet.com](http://www.henryscheinvet.com)).

- Prior to ordering controlled substances, a PI must establish an account with Henry Schein:
  - Contact: Tina Lauer | Ph: 855-724-3461 x 5518 Fax: 614-553-6856 | tlauer@henryscheinvet.com
  - Account set-up requires sending to Henry Schein:
    ✓ Completed Henry Schein customer form
    ✓ Copy of DEA license
    ✓ Copy of Certification of Exemption or practitioner's license
USE OF LABORATORY ANIMALS IN RESEARCH AND EDUCATION

- Once a PI has an account with Henry Schein Animal Health, they may place orders for schedule 3-5 controlled substances via Panther Express.

- The order should reference the PI's Henry Schein account number.

- Note that prescription drugs can be ordered through any vendor, but the PI must establish an account with that vendor.

- If a drug is not available from Henry Schein Animal Health, obtain a written certification that the drug is unavailable. Purchasing services will authorize the purchase from another vendor on a case-by-case basis.

- To order a Schedule 2 drug, the following must be forwarded to University purchasing (3309 Cathedral of Learning):
  - Completed purchasing requisition, noting the Henry Schein account number
  - DEA 222 form (provided when a DEA license is obtained or renewed)
  - Copy of the PI's currently active DEA registration
  - Copy of the PI's Certification of Exemption or PA practitioner’s license
8. Oversight

- DEA performs periodic audits of registrants
- IACUC reviews DEA records and storage of materials during semiannual inspections
- EH&S assures the proper security of controlled substances during laboratory inspections
- University employees who possess, sell, use or divert controlled substances or prescription drugs or devices in violation of Commonwealth or Federal laws will subject themselves to:
  - State and/or Federal prosecution
  - Potential University action regarding their continued employment

9. References for Further Information

- University Controlled Substance Guidelines: http://www.rcco.pitt.edu/ControlledDrugs
- Purchasing Guidelines: http://cfo.pitt.edu/pexpress/DEA.php
- EH&S Disposal Information: http://www.ehs.pitt.edu/workplace/waste.html
Critical Points for Performing Animal Research

1. ALL RESEARCH, TEACHING, OR TESTING PROJECTS THAT USE VERTEBRATE ANIMALS MUST BE FULLY APPROVED, in advance, by the University of Pittsburgh's Institutional Animal Care and Use Committee (IACUC).

2. ALL ANIMAL USE AND MANIPULATION MUST CONFORM TO IACUC APPROVED PROTOCOLS. All faculty, staff, or students must be listed on the applicable protocol, and are responsible for reading and following the procedures approved by the IACUC.

3. EMERGENCY VETERINARY MEDICAL CARE/CONSULTATION ARE AVAILABLE AT ALL TIMES, including after hours, weekends, and holidays. The veterinary service pager number for emergencies is: 412/958-5923.

4. CONCERNS ABOUT ANIMAL WELFARE AND/OR HUMANE TREATMENT OF ANIMALS should be brought to the attention of the Compliance Director at 412/383-2009, the Attending Veterinarian at 412/648-8950, or the IACUC Chair at 412/623-3233.

5. ALL PERSONNEL INVOLVED WITH ANIMAL PROTOCOLS MUST BE PROPERLY TRAINED OR EXPERIENCED with the proposed procedures for a particular species before initiation of the project. This applies especially to projects involving surgery.

6. PLANNING RESOURCES FOR ANIMAL BASED STUDIES ARE AVAILABLE through the DLAR. The use of such resources is encouraged, to producing the most humane and productive research efforts, grant preparation, and budgeting.

7. CONSIDERATION MUST BE GIVEN TO "ALTERNATIVE METHODS" when designing projects that use vertebrate animals. The intent of these considerations is to minimize the use of animals, and to reduce to the lowest levels possible any pain or distress in the animals. Such considerations should include the "three Rs" of Russell and Burch:
   a. Reduction in the number of animals used (implemented by good experimental design and statistical considerations)
   b. Refinement of techniques (familiarity with the techniques to be used through a good literature search or consultation with other investigators/veterinarians)
   c. Replacement of vertebrate animals with alternative research techniques or use of a lower phylogenetic model.

8. PROJECTS INVOLVING KNOWN OR SUSPECTED HAZARDS, SUCH AS HUMAN PATHOGENS, BIOHAZARDS, RADIOACTIVE MATERIALS, RECOMBINANT DNA OR CARCINOGENIC/TOXIC CHEMICALS IN ANIMALS MUST HAVE PRIOR APPROVAL by the appropriate committee or office before animals are ordered and experiments are executed. Further, a DLAR implementation meeting with the facility supervisor, DLAR veterinarian, and/or applicable safety offices may be mandatory prior to ordering animals.

9. AN OCCUPATIONAL HEALTH PLAN IS AVAILABLE TO EMPLOYEES WHO WORK WITH ANIMALS. In addition, employees injured on the job should be aware of University procedures for reporting such injuries/illness. (http://www.ehs.pitt.edu/biological/animal.html)