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SURVIVAL SURGICAL PROCEDURES

**ANIMAL SURGERY SUITES**

Amphibians
Cats
Chickens and other Avian Species
Chinchillas
Dogs
Ferrets
Fish
Gerbils
Goats/Sheep
Guinea Pigs
Hamsters
Mice
Nonhuman Primates
Pigs
Rabbits
Rats

**PURCHASE OF CONTROLLED DRUGS**

**ASSESSMENT OF PAIN IN LABORATORY ANIMALS**

**PROCEDURES IN COMMON LABORATORY RODENTS AND RABBITS**

**AGENTS AND METHODS OF EUTHANASIA BY SPECIES**

**FORMS**

**SUGGESTED READINGS**
Research Animal Resources

410-955-3273  
- Clinical calls for veterinary services  
- Technical assistance  
- Transportation of animals  
- Health Certificates

The Research Animal Resources office is open Monday through Friday from 8:30 a.m. until 5:00 p.m. The office is closed on weekends and all university holidays. After hours, leave an appropriate message in the voice mail system and the call will be returned as soon as possible. If you need veterinary assistance after hours, call the veterinary on-call pager: 410-283-0929

Associate Provost for Animal Research and Resources – Robert J. Adams, DVM  
Director of Research Animal Resources – Robert J. Adams, DVM  
Attending Veterinarian – Robert J. Adams, DVM  
Director of Laboratory Animal Management – Lindsay Barnes, BS, LATG

Molecular and Comparative Pathobiology

Laboratory Animal Medicine 410-955-3273  
- Research collaboration 410-955-3273  
- Academic programs 443-287-2953  
- Financial Manager 410-955-9783  
- Administrative Manager 410-955-9767

Director of Molecular and Comparative Pathobiology – Joseph Mankowski, DVM

Comparative Pathology

Comparative Pathology 443-287-2953  
- Diagnostic necropsy 443-287-2953  
- Research collaboration 443-287-2953

After 5 PM and on weekends and holidays, call the pathologist on call at 410-294-6220.

Animal Care and Use Committee

All studies, courses or training programs proposing the use of animals must be approved by the Institutional Animal Care and Use Committee

Office Telephone 443-287-3738  
Fax 443-287-3747  
Website http://web.jhu.edu/animalcare

Research Animal Resources

All laboratory animal resources of Johns Hopkins University are administered by Research Animal Resources (RAR) an operational arm of the JHU Provost’s Office with campus-wide responsibilities for animal care and use. Research Animal Resources evolved from Animal Services, a branch of the Department of Molecular and Comparative Pathobiology. A strong association continues between the RAR with the Department of Molecular and Comparative Pathobiology. RAR veterinarians perform their scholarly activities in JHU’s research and instructional programs through their academic appointments in the Department of Molecular and Comparative Pathobiology. Facilities included under RAR’s supervision are located in the School of Medicine, Bloomberg School of Public Health, Kimmel Cancer Center, and Homewood campus.

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Health, Research Farm, Bayview Medical Center, Homewood campus and The Johns Hopkins Hospital complex including the Cancer Research Building. Our program for the care and use of laboratory animals is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and the RAR managed facilities meet applicable regulatory guidelines.

RAR is responsible for the purchase of all research animals and the care of a majority of animals on the JHU campus. Animals are housed throughout the University and are maintained by RAR staff. Research Animal Resources is working towards the elimination of a small number of investigator maintained satellite animal colonies to enhance the uniformity of care and establish central accountability for the animals used in JHU research programs. The Research Farm, located 37 miles from the Medical Institutions, houses nonhuman primates socially in breeding groups for the production of Cercopithecine herpesvirus 1 (B-Virus) free offspring for use in the JHU research programs. The service objectives of RAR are to provide humane and scientifically appropriate care for the research animals at JHU in compliance with the Animal Welfare Act regulations of the U.S. Department of Agriculture and with the Guide for the Care and Use of Laboratory Animals. A further institutional objective is to provide this care as economically as possible through a centralized animal care staff, an institutional pool of cages and equipment, and wholesale purchase of supplies and equipment.

The RAR veterinarians implement the program of veterinary care and provide supervision of the RAR staff to identify, treat, and prevent intercurrent disease in research animals. The RAR veterinarians perform and/or coordinate the diagnostic activities for laboratory animal diseases which are provided free to investigators using animals under most circumstances due to the recovery of these costs through the per diem recharge system. However, some extraordinary veterinary costs related to conduct of specific experimental protocols may involve cost sharing by the Principal Investigator responsible for the project. Major areas of faculty expertise are in the fields of clinical laboratory animal medicine, retrovirus biology and comparative pathology. RAR veterinarians are available for consultation on animal research protocols, laboratory animal management, and development of grant applications proposing the use of animals. As faculty in the Department of Molecular and Comparative Pathobiology, they participate in the teaching of medical students and house staff, as well as graduate and undergraduate students. There are also departmental postdoctoral training programs in the fields of Laboratory Animal Medicine, Comparative Pathology and Retrovirus Biology.

Research Animal Resources, on behalf of the University, maintains an institutional membership in the American Association for Laboratory Animal Science (AALAS). This association publishes the journal, Comparative Medicine (formerly, Laboratory Animal Science) and The Journal of the American Association for Laboratory Animal Science (formerly Contemporary Topics). Contact Research Animal Resources if you are unable to get an article from either publication through the Welch library. The Institution also belongs to the National Association for Biomedical Research and the Foundation for Biomedical Research. These organizations act as spokespersons for the biomedical research community and provide education in the use of animals in research and teaching.

Alternatives to Animals in Research
The University shares society's concern that animal-based research should be appropriate and use no more animals than is necessary. Therefore, all scientists are urged to consider techniques that use minimal numbers of animals and are asked if they have investigated alternative methods to animal experimentation at the time of submission of research grant applications and animal use protocols for Animal Care and Use Committee. An excellent resource for achieving this goal is ALTWEB.

Use of Animals in Teaching Laboratories
The University Policy regarding the use of animals in teaching laboratories is that 1) the use of animals in teaching is appropriate, 2) students will not be required to participate in the use of animals in teaching exercises against their will, 3) when possible, alternatives to the use of live animals be explored, and 4) the guidelines established by the Institutional Animal Care and Use Committee should be observed in all courses using animals for teaching.
Laws and Regulations Applicable to Research Utilizing Animals

Animal Welfare Act

Scientists and the universities in which they carry out animal-based research or teaching fall under the “Animal Welfare Act” (7 U.S.C. 2132 et seq.). The objective of the legislation is to “effectively minimize the pain and discomfort of animals while under experimentation”. The Act covers nonhuman primates, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, and aquatic mammals and any other warm blooded animals used for biomedical research. Mice (Mus) and rats (Rattus) bred for research and birds are not covered by the Animal Welfare Act. In essence, the Act mandates unannounced inspections to ensure compliance with respect to humane care of animals used in research, their housing, and medical care including “the appropriate use of anesthetic, analgesic, or tranquilizing drugs, when such use would be proper in the opinion of the attending veterinarian at the research facility.” Annual reports are required, which the Department and the Institutional Animal Care and Use Committee staff prepares on behalf of the university. The Animal Welfare Act regulations (9 CFR Subchapter A, Parts 1, 2 and 3) are, for the most part, in congruence with the Public Health Service Policy outlined below. The Animal Welfare Act also mandates an annual review of all research by the Institutional Animal Care and Use Committee and semiannual inspections of facilities by the Committee. In addition, procedures that may cause more than momentary or slight pain or distress to the animals require a written narrative description of the methods and sources (e.g., the Animal Welfare Information Center of the National Agricultural Library) used to determine that alternatives to these procedures are not available. This narrative is required in the animal protocol, which must be submitted to the Institutional Animal Care and Use Committee for review and approval. In addition, there must also be written assurance that the activities do not unnecessarily duplicate previous experiments. The protocol must also describe the qualifications and training of the personnel with respect to the procedures to be performed. These points are all addressed in the instructions accompanying the Institutional Animal Care and Use Committee Protocol forms.

Enforcement of Animal Welfare Act regulations is carried out by veterinary inspectors from Animal Care, United States Department of Agriculture who make unannounced site visits every year to research facilities. Reports filed by these inspectors are available to the public under the Freedom of Information Act.

Public Health Service Policy on Humane Care and Use of Laboratory Animals

The Public Health Service policy requires that each institution receiving Public Health Services (PHS) funds (e.g. from NIH) for research involving animals submit detailed information in an Animal Welfare Assurance Statement of Compliance regarding the institution’s program for the care and use of animals. Johns Hopkins University has such an Assurance on file with the NIH Office of Laboratory Animal Welfare (OLAW).

Awardee institutions are required to identify an institutional official who is ultimately responsible for the institution’s program for the care and use of animals, and a veterinarian qualified in laboratory animal medicine who will participate in the program. Institutions are also required to designate clear lines of authority and responsibility for those involved in animal care and use in PHS-supported activities.

The policy defines the role and responsibilities of Institutional Animal Care and Use Committees and will enhance the involvement of such committees in all aspects of PHS-supported research at those institutions. The policy requires that Institutional Animal Care and Use Committees include an individual unaffiliated with the institution, a veterinarian who has program responsibilities and who has training or experience in laboratory animal science and medicine, a practicing scientist experienced in research involving animals, and a member whose concerns are in a nonscientific area.

The policy requires institutions to develop mechanisms to review and approve those sections of applications for PHS funds that relate to the care and use of animals (Vertebrate Animals Section) before PHS funds may be awarded.

Johns Hopkins University has assured the PHS that all uses of animals for research, testing or instruction will be in accordance with the Principles outlined below.
Association for the Assessment and Accreditation of Laboratory Animal Care International

The Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) is a “private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs”. AAALAC accreditation demonstrates that the facility has met minimum legal standards and has taken additional steps to achieve excellence in animal care and use.

Johns Hopkins University is fully accredited by AAALAC International. To maintain our accreditation, the IACUC submits an annual report. This report includes current personnel information and explains any changes that have been made to the animal care and use program. Voluntary scheduled site visits are held every three years to evaluate our program and offer advice and suggestions on improvement.

U.S. Government Principles for Use of Animals
(Federal Register, May 20, 1985, Vol. 50, No. 97, Office of Science and Technology Policy.)

These principles were prepared by the Interagency Research Animal Committee. This committee, which was established in 1983, serves as a focal point for federal agencies' discussions of issues involving all animal species needed for biomedical research and testing. The committee's principal concerns are the conservation, use, care, and welfare of research animals. Its responsibilities include information exchange, program coordination, and contributions to policy development.

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

The development of knowledge necessary for the improvement of the health and well-being of humans or animals requires in vivo experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible institutional official shall ensure that these principles are adhered to:

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.

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.

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 consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that 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 to conduct procedures on living animals. Adequate arrangements shall be made for their 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.

Failure to adhere to these regulations, policies and guidelines could lead to severe sanctions including civil and/or criminal penalties, loss of funding and university disciplinary action.

**Institutional Review of Animal Protocols**

**Protocol Submission**
Research activities which use live vertebrate animals require annual review and approval by the Institutional Animal Care and Use Committee. In order to accomplish this all investigators must submit experimental protocols on the Institutional review form. Periodically, updates are made to these forms. To prevent delays in processing the protocol, download and use the newest version of the protocol template from the ACUC website. After approval, the detailed protocol must be resubmitted and reviewed every three years; abbreviated annual renewal forms must be completed to assure continued access to animal use in the intervening two years. Requests for significant changes to previously approved protocols must be submitted on the appropriate amendment form. Links to all forms are included at the end of this book along with examples of the reminder letters that accompany them. Completed forms should be submitted to the Institutional Animal Care and Use Committee as indicated below.

The protocol review forms have been designed to provide the committee with the necessary information to adequately review the proposed animal work. The questions asked are required by both the Federal Animal Welfare Act regulations and Public Health Service Policy.

<table>
<thead>
<tr>
<th>All Schools:</th>
<th>Deadline:</th>
<th>Number of Copies:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutional Animal Care and Use Committee B122 Reed Hall</td>
<td>First business day of each month</td>
<td>1</td>
</tr>
</tbody>
</table>

**Institutional Animal Care Committee approval is necessary for all vertebrate animals used in testing, research, and training in the University.** All investigators will be required to provide protocol approval numbers at the time of ordering animals or arranging for billing. Amendments must be approved before the new procedures can be performed. Submission of an amendment is not sufficient; it must go through the review process and be approved. All individuals involved in the handling or procedures performed on animals must be included in the protocol.

**Committee Review of Protocols**
Protocols must be submitted by the first business day of each month in order for the Institutional Animal Care Committee to review it during the committee’s monthly meeting on the third Thursday of the month. Protocols will be assigned to individual members of the Committees who will act as primary reviewers. These reviewers will contact the investigator with any questions or suggestions before the meeting in order to facilitate approval of protocols. In this situation, the full committee grants approval for the protocol. Protocols can also be reviewed by the designated review system appointed by the Chair, who has the authority to approve the protocol, require modifications to secure approval, or request full committee review. For additional information on protocols, amendments, change in personnel, etc. contact the Institutional Animal Care and Use Committee.

**Grant Submission and Review**
*The following general guidelines are applicable in all Schools of the University.*
In constructing a Public Health Service (PHS) grant application, the principal investigator should include a section
devoted entirely to vertebrate animal use. After the investigator receives notice from the funding agency that his/her grant application is likely to be funded, he/she must submit an animal use protocol covering all the activities described in the vertebrate animal section for review and approval by the Animal Care and Use Committee. Animal protocol approval is no longer required prior to grant submission. However, an approved protocol consistent with the vertebrate animal section is required prior to awarding a grant. Investigators with good priority scores (likely fundable) on their grants are advised to submit an animal use protocol consistent with the vertebrate animal section of their grants as soon as they learn of their scores. This will allow sufficient time to review and approve their animal use protocols without delaying their funding.

PHS Grant Requirements for Use of Vertebrate Animals
Review of this section is required for all new and renewal PHS grant applications. The grant (Research Plan/or Vertebrate Animal Section) should contain all the information that the National Institutes of Health (NIH) has specified shall be included in this section, i.e.:

a. Provide a detailed description of the proposed use of the animals in the work previously outlined in the research design and methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

b. Justify the use of animals, the choice of species, and the numbers used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

c. Provide information on the veterinary care of the animals involved.

d. Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain and injury.

e. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association Guidelines for Euthanasia. If not, present a justification for not following the recommendations.

The vertebrate animal section should contain all the required information (a-e), or it will be deemed incomplete.

Other Agencies
Applications for funding from other agencies e.g. American Heart Association, often reflect different policies regarding Institutional Animal Care and Use Committee review; in many instances certification of committee approval must accompany the grant at time of submission.

Specific Requirements of Individual Schools of the University
School of Arts and Sciences:
All grant applications and the materials and methods and the Vertebrate Animal Section are reviewed by the Dean of Research and Graduate Education. The vertebrate animal section is compared to the cited approved protocols to insure that all proposed work has been reviewed and approved by the Institutional Animal Care and Use Committee. Investigators should provide copies of their approved animal use protocols together with their grant application. For further details contact the Sponsored Projects Officer, 237 Mergenthaler Hall (410-516-8841).

School of Engineering:
All grant applications, the materials and methods, and the vertebrate animal sections are reviewed by the Associate Dean for Research. Investigators should provide copies of their approved animal use protocols together with their grant application. For further details contact Research Projects Administration, W-400, Wyman Park Center (410-516-8668)

School of Medicine:
All grant proposals are submitted to the Office of Research Administration (129 SOM Administration) with the other required materials. The materials and methods, and the vertebrate animal sections are reviewed by Animal Care and Use Committee’s Training and Compliance staff.

School of Nursing:
All grant applications, the materials and methods, and vertebrate animals sections are reviewed by the Associate Dean for Graduate Education and Research, Bloomberg School of Public Health. Investigators should provide copies of their approved animal use protocols together with the vertebrate animal section of their grant application.
no more than one week before sign off is required. Call 410-614-3340 for additional information.

**Bloomberg School of Public Health:**
All grant applications, the materials and methods, and the vertebrate animals sections are reviewed by the Associate Dean for Graduate Education and Research. The Institutional Animal Care and Use committee will provide copies of approved protocols to the Dean’s office upon request.

**Protocol Records**
The original signed animal use protocol forms are retained by the Institutional Animal Care and Use Committee. Research Administration keeps copies of Vertebrate Animal sections submitted with grant applications, and an information sheet for each application, which records details of the Vertebrate Animal Section deficiencies and the protocol numbers cited for that application.

**Budgeting Animal Costs**
Research Animal Resources will be happy to provide purchase and care cost estimates. For this information please contact Animal Resources at 410-955-3713.

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**Training in Animal Care and Use**

Qualifications of investigators and the students and staff working with them are assessed by the Institutional Animal Care and Use Committee during review of protocols. Assessment of training is also monitored by the veterinary staff that makes regular rounds through animal areas to assure well-being of research animals in the facility. Training of individuals in the appropriate research animal techniques is provided in several ways.

All faculty, staff and students working with animals are required to complete the **online training** through MyLearning on animal care and use. The course explains the function of the Institutional Animal Care and Use Committee, federal and state laws as well as institutional policies governing the use of animals in biomedical research and the occupational health program for the care and use of animals.

Individuals working with animals are required to be familiar with the information contained in the handbook, **Use of Experimental Animals at Johns Hopkins University** (Blue Book). This handbook contains information about federal and state laws as well as institutional policies governing the use of animals in biomedical research and information about handling animals. In addition the animal care and use site ([www.jhu.edu/animalcare](http://www.jhu.edu/animalcare)) contains a wealth of information related to animal care and use. Contact the Animal Care and Use Committee office at 443-287-3738 for training and resources available to individuals using animals at Johns Hopkins University.

**Individual training:** Individuals using animals for the first time or employing unfamiliar techniques may be trained in these procedures by the scientific staff within the laboratory in which they are working or by the veterinary and technical staff of Research Animal Resources or the Institutional Animal Care and Use Committee training and compliance staff. When the Research Animal Resources staff is unfamiliar with the technique to be used, other individuals within the institution who can provide appropriate guidance are consulted. When necessary, individuals are referred to scientists and laboratories at other institutions. For unusual techniques, the Institutional Animal Care and Use Committee will require evidence of specialized training.

**Scheduled training:** The Institutional Animal Care and Use Committee schedules classes in rodent handling and rodent surgery several times throughout the year. For more information and to sign-up for a class, contact the Animal Care and Use office at 443-28-3738 or e-mail acuc@jhmi.edu.

**Library support:** The University maintains a wide range of publications describing proper use of animals in research. These are housed in the library of the Department of Molecular and Comparative Pathobiology and serve as a resource for individuals throughout the University. Included are: the American College of Laboratory Animal Medicine (ACLAM) series on Laboratory Animals, the MTM Laboratory Manual for Basic Biomedical Laboratory Animals, and a wide range of texts and journals on laboratory animal medicine and veterinary medicine.

**Courses:** Basic surgical training to medical students is provided in the course on surgical skills, which uses swine in
non-survival procedures. In addition, elective courses for staff and students offered by the Department of Molecular and Comparative Pathobiology include the following:

680.700 Molecular and Comparative Pathobiology Research Seminar Series. Offered September through June; Fridays at 1:00 p.m.
Description: One hour seminars given by faculty throughout the institution and outside guest speakers dealing with naturally occurring diseases of animals that relate to medical research and human disease, and with animal models of human disease.

680.701 Comparative Pathology and Genetically Engineered Mice. Offered fourth quarter; Even-numbered years. Prerequisites: Courses in biology, histology, and physiology or permission from course director. This is a course in comparative pathology and disease mechanisms for graduate students.
Description: Lecture and laboratory (microscopy) introduction to comparative pathobiology of the major organ systems. Pathology and pathophysiology themes that are shared across many species are covered, particularly those relevant to mouse genetic models and human disease.

680.703 Animal Pathology Laboratory. Offered all quarters. Credit to be arranged. Prerequisite: 680.701, or Pathology 300.600, or their equivalents.
Description: A limited number of persons may serve as prosectors on the animal pathology diagnostic service. This entails responsibility for gross and microscopic examination of diseased animals and tissues submitted for diagnosis by investigators within the institutions, by practicing veterinarians, and by the National Zoo in Baltimore. Complete necropsy and histopathology laboratories are available and prosectors work under close faculty supervision. Rotational assignments may vary according to schedules.

680.705 Introductory Course in Large Animal Surgery. Offered all year. Days offered will vary and the syllabus has yet to be determined.
Description: This course is designed to provide an opportunity for investigators, residents and students to gain hands on experience and training in large animal surgery and anesthesia, primarily using pigs and rabbits. The training will be done in tandem with other lab procedures and the course can be customized to fit the needs of the students. Students may gain experience in minimally invasive surgery, open surgery, suturing, instrument use and handling, catheter and/or sheath placement, intubation and maintaining animals under anesthesia.

680.711 Comparative Pathology Conference. Offered all quarters, Fridays 12:00; MRB, 8th floor conference room, 801
Description: This is a weekly one hour diagnostic slide conference focuses on the discussion of histologic and electron microscopic examples of unknown cases drawn from a wide variety of animal species. Cases are available for study during the week preceding the conference. Participants describe the cases, give differential diagnoses, and discuss etiology and pathogenesis with the guidance of faculty members.

680.712 Phenotyping for Functional Genetics. Offered 3rd Quarter, Wednesdays and Thursdays; MRB, 8th floor conference room, 801, 2:30-4:00.

187.620 Toxicological Pathology. Wednesdays and Fridays 3:00-4:50. Offered even-numbered years.
Description: Focuses on pathophysiology and pathologic responses of toxicity induced by toxins of the cardiovascular, pulmonary, reproductive, neurological, immune, and
gastrointestinal systems. A review of normal histology for the specific organ systems is compared to examples of acute and chronic toxicity to illustrate light microscopic and ultrastructural damage with correlation to altered physiology and function. The course integrates into each organ system studied a review of standard techniques used in toxicity studies including the use of animal necropsy, histology/pathology, various tissue molecular biological techniques, transgenic mice, and noninvasive physiological monitoring.

200.702 Molecular Biology- Fundamentals and Protocols. Offered in the Summer

**Occupational Health and Safety in Animal Care and Use**

**Introduction**
Johns Hopkins University is concerned about the safety and welfare of its faculty, staff and students. We are committed to alerting individuals to potential work-related health risks and counseling them on methods to avoid workplace hazards through a comprehensive occupational health and safety program.

**Safety Responsibilities**

**Individuals**
All faculty, staff, students and fellows are responsible for compliance with appropriate safety and health standards as issued by Johns Hopkins University. Faculty, staff, students and fellows are to follow safe work practices and report all unsafe conditions.

**Faculty and Supervisors**
Faculty and supervisors are the keystone of the Johns Hopkins University Safety Program. Faculty and supervisors train employees, fellows and students to develop and maintain safe work practices. Faculty and supervisors must frequently inspect the workplace to ascertain unsafe conditions. Faculty and supervisors should be aware that Johns Hopkins University has policies addressing disciplinary action for failure to comply with safety policies. Principal investigators are responsible for ensuring that all individuals working under their supervision have a safe working environment and are educated of the potential hazards associated with their jobs.

**Health, Safety and Environment**
The Department of Health, Safety and Environment is responsible for providing guidance and direction in all phases of the Johns Hopkins Safety Program. Health, Safety and Environment conducts safety inspections, and advises management of unsafe conditions or noncompliance with policy, regulations and standards. Health, Safety and Environment reports all of its activities to the Joint Committee for Health, Safety and Environment.

**Joint Committee on Health, Safety and Environment**
The Joint Committee on Health, Safety and Environment was established by the President of The Johns Hopkins University and the President of The Johns Hopkins Hospital to develop and enforce policies related to health, safety and the environment, and to ensure compliance with all applicable safety and environmental regulations. The Joint Committee on Health, Safety and Environment collaborates with the Institutional Animal Care and Use Committee to assure safety of individuals involved in the care and use of animals.

**Animal Exposure Surveillance Program**
All individuals (faculty, staff, fellows and students) who are exposed to animals, living tissues, body fluids, waste, bedding, their living quarters or equipment involved in the care and use of animals are required to participate in the Animal Exposure Surveillance Program (AESP). This program is managed by the Occupational Health Services, 98 North Broadway, Room 421 (410-955-6211). The program is described in detail later in this book. The AESP allows Johns Hopkins University to monitor your level of risk in handling animals, offer you appropriate prophylactic protection from diseases associated with animal handling, assess your current health status, and monitor your health during employment or training at Johns Hopkins University.

People with impaired immune function may be at increased risk from hazards associated with animal care and use.
Moreover, a large number of workplace chemicals, physical and biologic agents can damage the reproductive systems of both male and female workers, resulting in infertility, spontaneous abortion, developmental impairment or death in an embryo, fetus or child. These risks are discussed during AESP enrollment.

Identification and registration of individuals at risk
- Principal investigators and departmental administrators are responsible for forwarding the names of postdoctoral fellows and students with exposure to animals, body fluids, waste, bedding or equipment involved in the care and use of animals to Occupational Health Services. Human Resources will identify other staff during pre-employment screening. In addition, the Dean’s and Registrar’s offices from the various schools provide information about faculty and students eligible for enrollment in the program.
- Occupational Health Services conducts pre-employment medical examination and risk assessment.

Education
- Pre-employment orientation
- Encourage safe procedures
- Engineering controls and personal protective equipment e.g.: gloves, face masks, face shields, clothing, sleeve covers, caps, shoe covers, gowns
- Biosafety cabinets, fume hoods, dump stations, scavenging systems, fluid barriers, hand washing
- Using multiple people to handle large, heavy pieces of equipment

Health Monitoring
- Occupational Health Services (410-955-6211)
- Health, Safety and Environment (410-955-5918)

Post injury or exposure treatment. (5-STIX i.e. 410-955-7849)
- Occupational Injury Clinic in Blalock 139 (410-955-6433)
- Homewood Campus report to 6th floor Wyman Park Building (410-516-0450).
- Bayview Campus report to Asthma and Allergy Center, Room 2B.34 (410-550-2322).

Hazards associated with working with animals
- Physical e.g. bites, scratches, kicks, sharps, noise, ergonomic hazards, wet floors, electricity, steam and radiation.
- Biological e.g. viruses, parasites, bacteria, rickettsiae, fungi which can be transmitted between animals and humans (zoonotic agents).
- Chemical e.g. cleaning agents, anesthetics, laboratory chemicals: which may be flammable, explosive, corrosive, irritating or toxic. Always check Material Safety Data Sheets.

*Occupational Health and Safety in the Care and Use of Research Animals*, (1997) Institute of Laboratory Animal Research, National Research Council and *Biosafety in Microbiological and Biomedical Laboratories*, (1999) Public Health Service/CDC/NIH provides extensive information and references.

Hazardous materials require approval by Health, Safety and Environment (410-955-5918) before use in animal facilities. Investigators using biohazards or radioactive materials must contact Radiation Safety and the Director of Laboratory Animal Management (Lindsay Barnes 410-955-3273) to determine how to handle and dispose of animals, tissues, carcasses and bedding contaminated by these materials.

- Radioactive materials
- Infectious or oncogenic agents
  - Biosafety Office - 410-955-5918.
- Carcinogenic or toxic chemicals
  - Environmental Health Office - 410-955-5918.

Laboratory Animal Allergies
Allergic reactions to animals are among the most common conditions that adversely affect the health of people...
working with animals in research. Over 40% of people routinely working with animals develop allergic symptoms. More than 70% of people with pre-existing allergic disease eventually develop an allergy to laboratory animals over a period of 1-2 years, most commonly manifested as rhinitis, itchy eyes, and rashes. An estimated 10% of laboratory workers eventually develop occupationally related asthma, and related symptoms of coughing, wheezing, and shortness of breath, which can persist for months or years after exposure ceases.

Rats, mice, guinea pigs, rabbits and cats are probably among the most important inducers of allergies in laboratory animal workers. Allergens present in the urine, saliva, fur, dander, bedding and other unknown sources are aerosolized during handling of the animals, clipping hair, cage changing, dumping bedding and cleaning the animal rooms. Personal protective equipment such as laboratory coats, gloves, face masks, respiratory equipment, biosafety cabinets and dump stations reduce the risk of developing allergies.

Individuals who are already sensitized, for example due to allergies to domestic cats, are in the highest risk category. Laboratory animal workers should undergo screening to identify those at risk and participate in the monitoring program. If you develop symptoms of job related animal allergies contact Occupational Health Services at 410-955-6211.

Allergies are an important risk associated with animals. If you feel you may suffer from an allergy to the animals you work with, report to your supervisor and to the Occupational Health Services for your campus to obtain appropriate treatment. Allergies can usually be managed by a combination of medical management and workplace strategies. It's important to consult with Occupational Health Services to determine the cause of your allergy in order to manage it effectively.

The following practices may help reduce your exposure to animal allergens:

- When possible, perform animal manipulations in a ventilated hood or a biosafety cabinet. If this is not possible, a dust mask or surgical mask may be helpful.
- When you’re not working in a hood or cabinet, make sure that the animal room or other work area is adequately ventilated and that all the air handling equipment in the room is in good order. If there is doubt, your supervisor can ask Facilities to measure the number of air changes in the room. Animal rooms should deliver at least 10 air changes per hour.
- Don’t wear your street clothes when working with animals. Wear protective clothing.
- Reduce your skin contact with animals by wearing gloves, sleeve covers and long-sleeved lab coats.
- Wash your hands frequently. Wash hands, face and neck before leaving the work area.
- Avoid touching your hands to your face while working with animals and animal equipment.
- Use hair nets or wash your hair on leaving the facility. Allergens in the hair will result in significant exposure to others and may be carried home resulting in prolonged exposure.
- Keep cages and your work area clean.

Physical Hazards

Bites and Scratches

All animals are capable of inflicting bites and scratches. Small animals, such as rodents and rabbits usually deliver relatively minor wounds. Larger species like cats, dogs and nonhuman primates can inflict severe wounds. All bite and scratch wounds can become infected by the normal bacterial flora of the animal's mouth or toenails, or by bacterial flora from the individual's skin. To prevent bites and scratches use proper animal handling techniques. Protective garments, such as gloves, gauntlets and long-sleeved laboratory coats limit injury to the hands and arms. Contact Research Animal Resources (410-955-3273) for guidance on appropriate animal handling and restraining devices.

In case of a bite or scratch immediately wash the wound with plenty of soap and water, contact your supervisor and proceed to the Occupational Injury Clinic (410-955-6433). If you are bitten or scratched by a cat, dog, nonhuman primate or wild animal notify Research Animal Resources (410-955-3273) so appropriate veterinary follow-up measures and diagnostic procedures can be performed on the animal. Each lab working with nonhuman primates should have a monkey bite kit available. The kit includes instructions on what to do in case of a monkey bite or scratch. Monkey bite kits are available through the Research Animal Resources veterinary technicians (410-502-5068).
Splashes
In case of a splash or exposure to potentially infectious material involving your eyes, nose or mouth, flush the site with water for fifteen minutes, and proceed to the Occupational Injury Clinic for your campus.

Musculoskeletal disorders
Musculoskeletal disorders are syndromes characterized by discomfort, impairment, disability or persistent pain in joints, muscles, tendons or other soft tissues with or without physical manifestations. If you experience any of these conditions while performing your job, please report to the Occupational Injury Clinic for your campus. If you would like a proactive ergonomic assessment of your work site, ask your supervisor to contact the Department of Health, Safety and Environment.

Wet floors
Wet floors are a prominent physical hazard in animal areas. Do your part in promptly reporting or eliminating wet floor surfaces. If it is necessary to walk across wet floors, use extreme caution. Proper non-slip shoes or protective boots are recommended for environments that consistently have wet floor surfaces. When possible, post wet floor signs to alert coworkers and visitors of this hazard.

High Pressure Water and Steam
High pressure water and steam are physical hazards for animal handlers who utilize equipment such as autoclaves, power washers, and cage washers. Avoid skin contact with high-pressure water and steam. When unloading an autoclave, verify that the pressure is at zero prior to opening the door. Slowly crack open the door and allow the steam to gradually escape. Allow materials in the autoclave to cool for at least 10 minutes prior to removal and use heat resistant gloves as necessary.

Electricity
Electricity is an important physical hazard in the care and use of animals. Johns Hopkins University Policy prohibits use of extension cords, unless an emergency situation is declared by administration. Use caution with power equipment, radios and other electrical devices, particularly in areas with wet floors, and water or steam sources.

Radioactive Materials
All individuals using radioactive materials and/or animals containing radioactive materials must be registered with the appropriate Radiation Control Unit for your campus (East Baltimore Campus, 410-955-3712; Bayview Campus, 410-550-2423; Homewood Campus, 410-516-7308). Any lab using radioactive materials must contact Radiation Safety and the Director of Laboratory Animal Management (410-955-3273) to determine how to handle and dispose of animals, tissues, carcasses and bedding contaminated by these materials. The following guidelines should be adhered to:
1. Anyone handling a radioactive animal must wear gloves when handling a cage or animal marked as radioactive.
2. Cages containing radioactive animals must be clearly and appropriately labeled by the investigator with a “caution radioactive materials” label, identification of the radionuclide, date of administration and daily Geiger counter readings. If the exposure rates outside the cage exceed 2 millirem/hour, the cage must be shielded or moved to an area so that staff will not be exposed to a radiation level above 2 millirem/hr.
3. It is the responsibility of the researcher to collect any material from the animal or cage (e.g., bedding, waste, etc.) that may be contaminated, and place these materials in a radioactive materials drum.
4. Laboratory personnel and not animal service personnel are responsible for handling contaminated bedding and animal waste, surveys and decontaminating cages and equipment.
5. Once the animal is no longer radioactive, contact the Animal Care Supervisor of that facility. Animal care staff will then resume daily care of the animal.

The most commonly used radioactive substances used at Johns Hopkins University in animal research include carbon 11, iodine 123, iodine 125 and 2-fluoro-2-deoxy-D-glucose (FDG or F18). Ten to 12 half lives must pass before the animal is considered safe. However since very small amounts are used, once the Geiger counter is zero, the animal is considered safe, even if 10-12 half lives have not passed.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half Life</th>
<th>Time until safe</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-11</td>
<td>20 minutes</td>
<td>3-4 hours</td>
</tr>
<tr>
<td>FDG</td>
<td>110 minutes</td>
<td>18-22 hours</td>
</tr>
<tr>
<td>I-123</td>
<td>13 hours</td>
<td>4-6.5 days</td>
</tr>
</tbody>
</table>

Revised 1/6/09, updated 2/27/13, 10/20/15
Contact the radiation safety office for your campus for more information.

**East Baltimore Campus**
- It is the responsibility of the researcher to monitor the cage and associated equipment for radioactive contamination, and to clean all contamination before the cage and associated equipment is returned to Research Animal Resources.
- Any radioactive animal carcass with a completed disposal tag should be taken to the cold room in the Ross 11th floor penthouse and placed in the yellow barrels.

**Bayview Campus**
- Researchers must have an approved disposal plan for radioactive animal carcasses before radioactive material use. Radioactive animal carcasses cannot be transported to the East Baltimore Campus.

**Homewood Campus**
- All experiments involving use of radioactive materials in animals must first be approved by the Radiation Safety Officer.
- Only laboratory personnel trained by an authorized user are permitted to handle radioactively labeled animals.
- Facilities can not be turned over to Research Animal Resources for cleaning until certified free of removable contamination.
- Radioactive animal waste and bedding must be disposed of in special drums maintained by the Radiation Safety Office. In no case will radioactive waste be transferred to the regular animal facility trash. Regular animal facility caretakers are not permitted to handle radioactive materials.
- Radioactive animal carcasses and bulk radioactive tissues require special storage and ultimate disposal by a licensed vendor. Contact the radiation safety office to make arrangements.

**Biological Hazards**
Common exposure routes for infectious agents are inhalation of aerosolized agents, splash of infectious materials to the skin or mucous membranes, or exposure via needle sticks, cuts and other sharps injuries.

Any exposure to potentially infectious materials should be reported via the incident reporting protocol previously outlined. Exposure to the mucous membranes should receive on-site first aid by flushing with copious amounts of running water. Exposure to the skin should receive on-site first aid by washing the affected area with soap and water. After on-site first aid, proceed to the Occupational Injury Clinic for your campus:
- East Baltimore Campus, Blalock 139
- Bayview Campus, Asthma and Allergy Center, Room 134
- Homewood Campus, Employees, Wyman Park Building, 6th floor
- Homewood Campus, Students, AMR II, Student Health Services

**Blood borne pathogens**
All individuals who could be exposed to human tissues and/or bodily fluids must complete annual training in Blood Borne Pathogens, mandated by OSHA and Johns Hopkins.

Johns Hopkins University Blood Borne Pathogen Exposure Control policy requires prompt reporting of exposure to human blood borne pathogens. Call 410-955-STIX (410-955-7849) to report exposures. The Johns Hopkins STIX Hotline is a 24-hour hotline staffed by an infectious disease specialist. If your exposure is high risk for blood borne pathogen infection, post exposure prophylaxis may be recommended. For example, medications have been shown to be effective in reducing the risk of HIV infection if initiated within 1-2 hours of exposure.

All faculty and staff with exposure to human blood borne pathogens will be entered in the Johns Hopkins University Blood borne Pathogen Exposure Control Program upon hire or upon initiating work with blood borne pathogen containing materials. All persons entered in this Program are offered the hepatitis B vaccination at no charge. The vaccination is obtained from the Occupational Health Services for your campus.

The basis of Standard Precautions is to treat all human specimens and primate specimens as infectious. Also treat all human subjects and nonhuman primates as infectious.
The highest risk of infection from blood borne pathogens occurs from needles and sharps. Sharps are defined as any item that can puncture human skin or a red trash bag. Needles and sharps are never to be discarded directly into the general waste stream or red trash bags. All needles and sharps must be discarded directly into approved sharps containers. Approved sharps containers must be placed in all areas where sharps may be utilized or generated. Filled sharps containers must be properly secured prior to disposal, and are disposed in red bags or biohazard boxes lined with red bags, as appropriate for your building.

Hand washing
Hand washing is the most effective way to prevent infections to you and coworkers. All animal areas should be equipped with a hand sink that is stocked with liquid soap and paper towels. Wash your hands often, using soap and water.

All infectious materials and all contaminated equipment or apparatus should be decontaminated before being washed, stored or discarded. Autoclaving is the preferred method for decontamination and sterilization. All areas and equipment involving any contact with sheep or goats or products of conception from sheep or goats shall be cleaned and disinfected on a regular basis and immediately after each operation. Transport carts shall be decontaminated after use.

The containment of infectious agents is performed according to the applicable Biosafety level. Biosafety Level I generally involves agents of no known or minimal potential hazard to laboratory personnel and the environment. Biosafety Level II includes all Biosafety Level I practices plus partial containment equipment such as biological safety cabinets, protective barriers such as lab coats, gloves, and face protection, and limited access to the laboratory. Biosafety Level II work involves agents of moderate potential hazard to personnel and the environment. Biosafety Level III is designated for research utilizing indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by the aerosol route. For more information read the Biosafety in Microbiological and Biomedical Laboratories 4th edition publication.

Zoonotic Diseases

Zoonotic diseases are those diseases of animals capable of infecting humans. A number of zoonotic diseases are of potential hazard in institutions conducting animal based research. Such diseases are rare in rodents, rabbits, cats and dogs raised indoors for research purposes. There is a higher possibility of contracting a zoonotic disease from random source cats, dogs, farm animals, nonhuman primates and animals obtained from the wild (e.g. wild rats, prairie dogs, woodchucks).

Humans may also become infected with agents introduced into animals for research purposes. All such biohazardous research must be registered with Health, Safety and Environment. You must be instructed by your employer in how to handle animals so infected. In case of injury or unexplained illness associated with such animal use, inform the medical personnel treating you of the possibility of infection with that agent.

Toxoplasmosis

Toxoplasmosis is caused by a protozoan parasite, Toxoplasma gondii. The potentially infective form of the parasite (oocyst) occurs in the feces of infected cats. Indoor raised laboratory cats are unlikely to be infected with T. gondii. It takes 2 to 5 days after the feces are passed for the oocyst to become infectious. Other animals, including man (and cats), become infected by eating material contaminated by infected cat feces, or by eating raw or improperly cooked tissue from other infected animals and from contaminated soil during gardening.

In humans, this disease is usually quite mild and may be mistaken for a simple cold. There may be a slight fever, mild headaches, general tiredness and enlarged lymph nodes. Rarely heart, pulmonary or liver disease, blindness or damage to the nervous system may occur. Individuals with a compromised immune system may develop serious illness. Transmission of the infection from a pregnant mother to her fetus sometimes results in severe birth defects, stillbirth or miscarriage.

Toxoplasmosis is easily prevented by avoiding exposure: effective pest and vermin control programs, gloves, masks, protective clothing, hand washing and do not eat, drink or apply make-up in animal areas. Pregnant women
should avoid working with cats, especially handling used cat litter or changing litter boxes. There is no vaccine to protect humans from this parasite.

**Rabies**

Rabies is a relatively rare and devastating viral disease, which can result in severe neurologic problems and death. Most cases of rabies occur in wild animals although any mammal can contract the disease. Rabies is usually transmitted from infected mammals (rabid cats, dogs or wild animals) to humans through bite wounds. Rarely, infection may occur if a scratch or abrasion is contaminated with saliva from an infected animal. Injuries sustained during endotracheal tube insertion should be treated as though it were a bite. Infected animals may shed the virus in saliva before the visible signs of illness appear and the virus can remain viable in frozen tissues for an extended period. Aerosol transmission is considered unlikely. Contact with bedding, cages or feed and water bowls does not constitute an exposure. Purpose bred dogs and cats raised indoors for research purposes by commercial suppliers are considered a low risk for transmitting rabies because they are not normally exposed to infected wild animals, and are usually vaccinated against rabies. Dogs purchased from Class B vendors have a slightly higher risk of being infected since the history of the dog is not known.

Animals are infected by being bitten by a rabid animal. The virus enters the skin and travels along peripheral nerves to the spinal cord. Once in the spinal cord, the virus ascends to the brain and then to the salivary glands. During the time the virus is migrating through the tissues and nerves, it is not infective. The animal becomes able to transmit rabies when the virus enters the salivary glands. This process of moving from the bite wound to the salivary glands can take weeks, depending on the site of the bite (distance from the salivary glands). Just because an animal is not showing clinical signs at the time of the bite, does not mean it is not infected with rabies. It just means that it cannot spread the disease at the time of the bite.

The clinical signs shown by animals infected with rabies are variable but may include: personality change for example a friendly animal may appear to have become shy or it may be restless, excitable, or aggressive; depraved appetite (animal eats strange objects); change in vocalization, inability to eat or drink, and excessive salivation due to paralysis of the laryngeal muscles; and incoordination or convulsions. These signs develop when the infected animal is actively shedding the virus.

Following a bite, immediately wash the wound with plenty of soap water and seek medical attention at the Occupational Injury Clinic. In addition Research Animal Resources (410-955-3273) should be notified. The animal should be kept alive, quarantined and monitored by veterinary staff for 10 days. If the animal was shedding rabies virus at the time of the bite, it will die within 10 days. Alternatively, the animal should be euthanized and the head submitted to the Maryland State Health Department Laboratory for rabies evaluation. Employees at potential risk are notified every two years that they need to come to Occupational Health Services to have blood drawn for a titer. If the titer is low, the employee will be notified and must receive a booster vaccination. You need to get a post-exposure booster even if you were vaccinated before.

**Tuberculosis**

Tuberculosis is a chronic disease caused by bacteria in the genus Mycobacterium. The disease is usually localized in the lungs but other organs can also be affected. The main source of infection for humans in the United States is other infected humans especially in crowded conditions and in hospital settings. This disease is highly infectious in a nonhuman primate colony often with a fatal outcome. The most common source of infection for monkeys is from infected humans or other monkeys.

For protection of the animals and individuals who work with them, monkeys are routinely tuberculin tested. At six-month intervals all individuals who work with nonhuman primates are notified of the time and place for tuberculin testing. Wearing personal protective equipment like facemasks, gloves and laboratory coats when handling or working with monkeys reduces the risk of exposure.

**Diarrheas associated with nonhuman primates**

Nonhuman primates are potential sources of diarrhea-causing organisms for humans. Animals may carry the organisms without showing signs of disease. The most common diarrhea-producing organisms seen in the nonhuman primates that are in this institution are *Entamoeba histolytica* (the cause of amoebic dysentery), Shigella (the cause of bacillary dysentery) and Campylobacter. Disease caused by these organisms may be severe (diarrhea containing blood and mucus) or less severe with varying degrees of fever, abdominal cramps and loose stools. If you develop diarrhea, inform the physician treating you that you work with nonhuman primates.

Revised 1/6/09, updated 2/27/13, 10/20/15
Minimize chances of such infections by using personal protective devices such as gloves, face masks, shoe covers and laboratory coats whenever you are in contact with nonhuman primates. Thoroughly wash your hands after working with primates and do not eat, drink, or apply make-up when working with nonhuman primates.

Cercopithecine herpesvirus 1 (Herpes B) in Macaque monkeys
All macaque monkeys (e.g. rhesus [Macaca mulatta], cynomolgus [M. fasicularis], pigtail [M. nemestrina]) are potential carriers of Cercopithecine herpesvirus 1 (Herpesvirus-B, Herpes B, Herpes simiae, B virus) and **should be treated as though they are infected with this virus**. This virus is not found in non-macaque species of monkeys such as the African green, squirrel, or baboon. In macaques, Herpes B sometimes causes cold sore like lesions but for the most part there are no obvious problems in the monkey. Though very rare, human infection is usually fatal or produces severe and lasting neurologic disease.

Humans become infected following a **bite or scratch, splashes or contact with tissues, body fluids or excretions**, and by **injury** from a cage or similar item contaminated with material from an infected monkey. Open wounds can also act as an exposure route. To prevent potential exposure use personal protective devices like **facemasks, face shields, gloves and long sleeved laboratory coats**. Unless absolutely necessary, never handle an awake macaque. Use a squeeze cage and a restraint drug such as ketamine to sedate the primate before handling. Be extremely cautious when working near the head or close enough for a monkey to grab you. Treat all monkeys as though they are infected with Herpes B virus.

If you receive a **bite or scratch wound from a monkey** or an injury from a piece of equipment with which the animal or animal tissues has had contact, **STOP WORK, return the monkey to its cage, and institute first aid immediately**. Each primate housing area and laboratory has been equipped with a "**Monkey Injury Kit**". Follow the instructions that are inside the kit. You should familiarize yourself with them in advance. Following initial first aid, go to the **Occupational Injury Clinic**. Notify Research Animal Resources of the identification number of the monkey and its location so that a veterinarian can examine the animal and take appropriate steps to ascertain the potential hazard. Monkey Bite Kits have expiration dates. Labs must make sure their kits are up to date. Contact Research Animal Resources veterinary technicians (410-502-5068) to request kits.

Experimentally induced infections
Simian Immunodeficiency Virus (SIV) is related to the Human Immunodeficiency Virus (HIV). This virus does not occur naturally in the primates currently in use at Johns Hopkins University. Any occurrence of this virus in a primate at this institution is a result of a deliberate introduction of the virus into the primate by an investigator. Such primates are clearly identified.

Individuals working with this virus or monkeys deliberately infected with the virus must follow guidelines that have been developed by the Centers for Disease Control and Johns Hopkins University. The guidelines include working under BSL2, and in some cases BSL3, conditions. In the event of a suspected exposure, follow procedures described for Cercopithecine herpesvirus 1 (Herpes B). A prophylaxis protocol will be instituted through Occupational Injury Clinic following exposure (call 5-STIX) and anti-retroviral therapy will be offered.

Other infectious agents that could be used include measles, vaccinia virus and Shigella.

Q-fever
Q-fever is caused by the rickettsia, *Coxiella burnetti*. Domestic ungulates such as sheep, cattle and goats are reservoirs of infection for humans and shed the organism in placenta, amniotic fluid, urine, feces and milk. Infection is acquired by working with these animals or their products or from laboratories working with *C. burnetti*. The illness is generally mild in people, however, hepatitis is a common sequel and endocarditis is an uncommon but frequently fatal complication. The disease should be diagnosed and treated promptly to minimize risk of complications. Currently there are no approved vaccines.

Laboratories working with sheep and goat, especially if pregnant, can reduce the risk of human infection with Q fever. Personal protective equipment can reduce exposure and include full body protective clothing including wraparound or solid front gowns or uniforms, head cover, gloves, boots, and a mask.

Contagious ecthyma (ORF)
The disease is caused by a poxvirus endemic in sheep and goats in the United States. All age groups are affected.
ORF produces proliferative, pustular encrustations on the lips, oral cavity, nostrils and urogenital openings of animals. The disease is transmitted to humans through direct contact with the lesions or contact with material from infected animals. Humans usually develop solitary lesions on the hand, arm or face. Lesions may be maculopapular, pustular, or proliferative. While they can resemble abscesses, lesions should not be lanced. Lesions generally regress spontaneously.

Chemical Hazards
The Johns Hopkins University Hazard Communication Program gives employees a means to find information about the hazards associated with any material in their workplace. Material Safety Data Sheets (MSDS) are fact sheets that summarize information about the hazards, handling procedures, emergency first aid and required protective equipment regarding each substance. Health, Safety and Environment has MSDS sheets for all substances used at Johns Hopkins. Should you have a concern or question about any substance in your workplace, discuss the situation with your supervisor. If the supervisor is unable to answer your questions, contact Health, Safety and Environment.

Labeling
Labeling is an important aspect of the Hazard Communication Program. All containers must have a label of the common name in English to identify the contents.

Cleaning agents
Cleaning agents are a common form of chemical utilized by animal handlers. All cleaning agents must be stored in labeled and tightly capped containers at all times. Consult the product label or the MSDS for appropriate protective equipment when handling cleaning agents. Always wear a face shield and gloves when handling and dispensing concentrated cleaners.

Corrosives and Flammables
Corrosive materials are the most common form of toxic substances found in a typical laboratory and are frequently used in animal facilities. These include strong acids, strong bases and oxidizing agents. When handling corrosive substances ensure that skin, face and eyes are protected by clothing, laboratory coats, aprons, eyewear and/or face shields.

Flammable materials are clearly labeled as such on the product label. All flammable materials not in immediate use should be stored in an approved flammable materials storage cabinet. Existing approved cabinets have doors which positively latch. Newly acquired flammable material storage cabinets must have doors which self-close and self-latch.

Anesthetic agents
Anesthetic agents have long been associated with health hazards. Chronic exposure to these agents may have possible effects on the liver, kidney, nervous system and reproductive system. Engineering controls, such as systems that scavenge waste gases from the source are the best methods to control these hazards. The use of ether is highly regulated at Johns Hopkins University and requires pre-approval by Health, Safety and Environment.

Excess chemicals
Excess chemicals should be disposed of through the Johns Hopkins Hazardous Material Disposal Program. Call Health, Safety and Environment (410-955-5918) for the nearest chemical collection area. Never place chemicals in the standard waste stream or in the biohazardous waste stream. Excess chemicals should not be labeled as “waste”. The Department of Health, Safety and Environment makes the determination of what is hazardous waste.

In the event of a spill of a hazardous material which is chemical in nature:
1. Evaluate the spill. Are the materials corrosive, flammable, toxic or explosive?
   A. Identify all materials by common name.
   B. Estimate how much is spilled.
   C. Evaluate the degree of danger to patients, staff or visitors.
   D. Evaluate the degree of danger to equipment or property.
2. Contain the spill.
3. If the spill cannot be contained, evacuate the area. Also evacuate the area if the spill is likely to produce irritating odors, or flammable or explosive vapors.
4. Spills of innocuous material or small amounts of acids, bases and flammable material can be cleaned up by lab personnel or properly equipped staff in the area.
5. All spills of toxic or explosive materials and large spills of corrosive or flammable material will be cleaned by Health, Safety and Environment. Immediately call the emergency number for your campus.
   - East Baltimore Campus 410-955-4444
   - Bayview Campus 410-550-2424
   - Homewood and other buildings 911

If your skin or eyes are exposed to a chemical, flush the affected area with large amounts of running water. After on-site first aid, proceed to the Occupational Injury Clinic for your campus.

Research Registration Programs
In order to control and monitor biological hazards in the work environment, Johns Hopkins University has established research registration programs in accordance with federal regulations and guidelines. It is the responsibility of the principal investigator to assure that individuals working with the registered agents and materials are appropriately trained and that the protocols are conducted in compliance with Johns Hopkins University policies.

Registration of Research with HBV, HIV or SIV
Experimental animal studies involving the use of human Hepatitis B Virus (HBV) or Human Immunodeficiency Virus (HIV), suspected HIV, Acquired Immune Deficiency Syndrome (AIDS) associated retroviruses or Simian Immunodeficiency Virus (SIV) are not to be initiated without prior approval from the Institutional Animal Care and Use Committee and the Department of Health, Safety and Environment.

Research with Animals
Federal laws and regulations require that all activities involving animals be approved by the Institutional Animal Care and Use Committee prior to initiation of those activities.

Registration of Research with Pathogenic and/or Oncogenic Material
It is the responsibility of each principal investigator to register with Health, Safety and Environment all biohazardous agents and materials presently in use for investigative research and for all agents maintained in stock culture collections for research and/or teaching purposes.

Registration of Research with Recombinant DNA
All principal investigators conducting recombinant DNA research are required to register such protocols with the Department of Health, Safety and Environment and the Institutional Biosafety Committee. Research involving recombinant DNA requires strict adherence to the most current NIH guidelines.

Incident and Injury Reporting
It is the policy of Johns Hopkins University that all incidents which result in an injury to faculty, staff or students be appropriately documented and reported. In the event of a work-related incident:
1. Promptly report to your supervisor.
2. Fill out a Report of Incident form.
3. Proceed to the Occupational Injury Clinic for your campus:
   - East Baltimore Campus, Blalock 139, 410-955-6433
   - Bayview Campus, Asthma and Allergy Center Room 2B.34, 410-550-2322
   - Homewood Campus, Employees, 6th Floor Wyman Park Building, 410-516-0450
   - Homewood Campus, Students, Student Health Services, AMR II, 410-516-8270

Medical Emergency
If the accident or injury is life threatening, or if the injured individual believes his or her injury is of an emergent nature, call the emergency number for your campus:
   - East Baltimore Campus 410-955-4444
Emergencies
All faculty, staff and students should be aware of the emergency protocols for their campus. In the event of fire or other emergency:
1. Remove anyone from immediate danger.
2. Close the door to confine smoke, fire or hazardous conditions.
3. Pull the nearest fire alarm box.
4. Call the emergency number for your building.
   - East Baltimore Campus 410-955-4444
   - Bayview Campus 410-550-2424
   - Homewood Campus 911
   - Other Johns Hopkins buildings 911

Part of the emergency response protocols for Johns Hopkins includes hazard-warning signage. This yellow placard is required to be posted at the entrances to all laboratories and research areas to indicate the hazards contained therein. These yellow warning placards must contain the names and emergency telephone numbers of two individuals who are familiar with the hazards contained within the area. Emergency responders may refuse to enter a placarded area prior to discussing the hazards contained therein with the emergency contact individuals. It is the responsibility of the principal investigator or the area supervisor to include the emergency contact information on the yellow placards. If your area does not have the proper warning signage at its entrances, contact Health, Safety and Environment.

If you have any questions regarding this information contact Health, Safety and Environment. Telephone 410-955-5918. Address: 2024 East Monument Street, Baltimore, MD 21205.

Animal Exposure Surveillance Program
I. Purpose
The purpose of the Animal Exposure Surveillance Program (AESP) is to provide relevant health and safety information related to the care and use of animals; occupationally indicated immunizations; and clinical evaluation and treatment for individuals with animal related injuries or illnesses.

II. Eligibility
All Johns Hopkins University faculty and staff are required to participate in this program if they are involved in the direct care of animals or their living quarters; or have direct contact with animals (live or dead), their viable tissues, body fluids, or waste.

III. Identification and Enrollment
Investigators are required to list all individuals working with animals on the Institutional Animal Care and Use Protocol Form. Anyone working with animals must complete the Animal Exposure Surveillance Program questionnaire and submit it to Occupational Health Services before they can be added to an animal protocol. If questions arise from the information obtained in the questionnaire (e.g., animal allergies, pregnancy etc.), the individual will be contacted by someone in Occupational Health Services to discuss options for alleviating problems that may occur from working with animals.

Occupational Health Services also enrolls eligible workers when the individual, who is undergoing a pre-placement medical evaluation, indicates their involvement in either utilizing animals or their viable tissues.

At the completion of their assessment, information is entered into the AESP database which contains names, dates and times of appointments, immunizations recommended or required and immunizations administered. This database is maintained by Occupational Health Services and updated monthly.

IV. Program Organization
The surveillance program is subdivided into four broad categories: (1) small animal e.g., rodents; (2) large animal
e.g., rabbits, cats, dogs, livestock; (3) nonhuman primate e.g., marmosets, monkeys; and (4) nonhuman primate tissues.

V. Services Offered to All AESP Participants and those working with Small Animals

A medical evaluation, which includes an occupational medical history, safety and health counseling, tuberculin skin testing, and appropriate immunization(s) is performed.

The occupational medical history includes a review of the functional demands and environmental factors associated with the proposed position, the type of animal(s) contacted, other potential work-site health hazards and the individual's medical history.

The participant is counseled regarding the blood borne pathogen exposure control program and the Johns Hopkins Policy requiring medical evaluation and treatment in the Occupational Injury Clinic for all occupational injuries and illnesses. The participant is also given information and handouts regarding relevant zoonoses based upon the animals used at the work site and information on safety and health risks for animal handlers.

Tuberculin skin testing (PPD) is administered if there is no history of a prior positive test. A chest radiograph is also required if the participant (1) offers a history of a prior positive test and cannot provide documentation of a normal chest radiograph two years or more following the discovery of the positive tuberculin skin test; or (2) is discovered to have a positive tuberculin test reactor.

The participant is given a booster dose of tetanus/diphtheria (Td) toxoid, if clinically indicated.

Illnesses – infections

Employees are counseled by the Occupational Health Services practitioner during the enrollment evaluation to report any gastrointestinal, respiratory, or dermal illness with signs or symptoms which resemble those occurring in the animals for which they care. Some of the agents responsible for infections in laboratory animals are capable of infecting humans.

Illnesses – allergies

During the AESP enrollment, Occupational Health Services screens employees at risk for developing work related allergies by requesting a history of pre-existing allergies, asthma, seasonal rhinitis, or eczema. Enrollees are advised of the availability of the Occupational Injury Clinic and are encouraged to seek evaluation and treatment if they develop symptoms suggestive of a work related allergy.

VI. Large Animals

A participant with large animal contact may receive the following services in addition to those listed in Section V.

(A) Rabies immunization

Rabies immunization is provided to employees who work with the rabies virus; have direct contact with quarantined animals potentially infected with rabies; work with potentially infected animal body organs or perform post mortem examinations on selected animals with a history of poorly defined neurological disorders; capture or destroy wild animals as part of employment with Johns Hopkins; or, inspect facilities where the rabies virus is used.

(B) Serologic testing for toxoplasmosis

A toxoplasmosis antibody titer is obtained for female employees of childbearing capacity who anticipate occupational exposure to cats or their feces. Females of childbearing age who may be exposed will be informed of their antibody status and provided information regarding the risk of exposure and the methods to eliminate risk of contracting this disease.

(B) Q Fever

Counseling and Treatment

Employees at risk of exposure to Q fever include those who have direct involvement with the organism Coxiella burnetii in a research capacity, or handle or use products of parturition or material contaminated by them (e.g., placentas, amniotic fluid, aborted feti) from sheep or goats.

At the time of the pre-placement medical evaluation, the participant is evaluated for the likelihood of developing chronic sequelae should they acquire Q fever. Employees with valvular or congenital heart defects or immunosuppression are advised of the potential risks involved, and medical clearance for duty will be determined.
Occupational Infection
The incubation period averages 20 days, with a range from 14 to 39 days. Signs and symptoms of acute infection include the sudden onset of severe headache, high spiking fever to 104°F or greater, chills, and myalgia. The patient may present with pneumonitis or clinical hepatitis. Treatment is initiated as soon as diagnosis is suspected. Serologic confirmation of the diagnosis is accomplished three months later using enzyme immunoassay (EIA), testing of serum samples obtained at the time of initial report, at two weeks and every 30 days from that day for three months. The employee's work status depends upon the severity of symptoms. Human to human transmission of Q fever has not been documented.

VII. Nonhuman Primates
Participants working with or caring for nonhuman primates and those workers performing necropsies on nonhuman primates are offered the following services in addition to those listed in Section V.

(A) Tuberculosis Screening
Tuberculosis is a zoonotic disease which is difficult to detect in nonhuman primates and spreads rapidly in nonhuman primate colonies. Because there is no effective treatment for this infection in nonhuman primates, infected animals are euthanized to control the spread of the infection. Due to the devastating consequences of tuberculosis for nonhuman primates and associated research projects, special precautions are taken to reduce the risk that workers involved in the use and care of these animals will infect them with tuberculosis.

If the participant has a history of a previous positive reaction to a tuberculin skin test, further skin testing is not performed. A Tuberculosis Health Questionnaire is administered and the completed form is filed in the employee's Occupational Health Services medical record. A chest radiograph is obtained if the employee's responses to the questionnaire suggest active pulmonary tuberculosis or the employee cannot provide documentation of a normal chest radiograph following the discovery of the positive reaction. A chest radiograph is obtained if the participant received inappropriate chemoprophylaxis or treatment.

Participants working with nonhuman primates who do not have a history of a prior positive reaction to a tuberculin skin test receive one tuberculin skin test on enrollment. A second skin test is administered one to two weeks after the initial test. If the first tuberculin skin test is positive, a medical history is obtained for symptoms suggestive of active pulmonary tuberculosis and a chest radiograph is obtained. If the individual did not have a documented negative skin test in the preceding 24 months (i.e., the test result does not represent a tuberculin skin test conversion), and there is neither clinical nor radiographic evidence of active pulmonary tuberculosis, the employee is counseled, referred for further care as indicated, and medically cleared for duty. If the employee had a documented negative skin test in the preceding 24 months and there is no radiographic evidence of active pulmonary disease, the employee is medically restricted from contact with live nonhuman primates until appropriate medical treatment has been received for at least three days. Occupational Health Services offers prophylaxis. If there is clinical or radiographic evidence of active pulmonary tuberculosis, the employee is medically restricted from returning to work. This restriction is not removed until the individual provides documentation establishing that the clinical or radiographic findings can reasonably be attributed to a condition other than active pulmonary tuberculosis. The worker is not cleared to return to the work place until the Occupational Health Services medical director is reasonably convinced that the individual does not represent a health risk to others. Continued compliance with treatment is monitored by Occupational Health Services. If the medical recommendation is that the employee not work with live nonhuman primates, or not return to work, the employee, supervisor, and Human Resources are notified the day the decision is reached. If the initial tuberculin skin test is negative and the second test is positive, the response is indicative of a prior infection (booster phenomenon) and the course of action is as described in Section VII. If both of the tuberculin skin tests are negative, and there are no other medical contraindications, the employee is medically cleared for work.

Evaluation of persons sustaining a potential work-place exposure to tuberculosis is conducted as described in the Tuberculosis Surveillance Program.

(B) Rubeola (measles) screening
Rubeola is one of the most frequently reported viral diseases of nonhuman primates. Due to the potential personal and public health consequences associated with rubeola infection, all employees working in rooms containing nonhuman primates must have laboratory evidence of protection to rubeola.
(C) Retrovirus testing
Simian Immunodeficiency Virus (SIV) infections occur naturally in African Green monkeys, baboons, sooty mangabeys, and chimpanzees. The infection commonly persists without any clinical manifestations. Several species of the genus Macaca (e.g., rhesus, cynomolgus) are highly susceptible and die following experimental or colony acquired SIV infection. To date, there have been three documented occupational infections with SIV. The medical significance of these infections is not yet clear.

Type D retroviruses (simian retroviruses, SRVs) may infect rhesus, cynomolgus, squirrel, pig-tailed, bonnet, and langur monkeys. It has not been convincingly demonstrated whether humans have been infected with type D retroviruses.

Routine serologic testing for SIV/HIV-2 is offered for participants using or caring for nonhuman primates that are known or suspected to be infected with SIV/HIV-2. Individuals exposed to SIV/HIV-2 must participate in the Johns Hopkins Bloodborne Pathogen Program. Individuals exposed to SIV/HIV-2 will be offered Post Exposure Prophylaxis as part of the Bloodborne Pathogen Program.

(D) Viral Hepatitis Screening
Nonhuman primates (e.g., rhesus, cynomolgus, African green, tamarin, and owl monkeys and chimpanzees) are used in research on hepatitis A, B, C, D, E, and G. Employees working with these research animals may be at risk for exposure to the virus used in the research. Participants working with nonhuman primates experimentally infected with hepatitis A or B are offered the appropriate vaccine(s).

(E) Rabies
Rabies immunization is offered to employees working with animals at risk for rabies.

(F) B-virus (cercopithecine herpes virus 1) testing
Injuries involving neurologic tissue or either oral or ocular secretions of rhesus, cynomolgus and other macaque monkeys (e.g., pig-tail, and stump-tail monkeys) very rarely result in human infection with B-virus. However, due to the extreme morbidity and mortality of this infection in humans, special effort is taken to ensure prompt medical evaluation and first aid following a potential exposure to B-virus.

VIII. Nonhuman Primate Tissues
Participants who work with nonhuman primate tissues may receive the following services in addition to those listed in Section V.

(A) Periodic tuberculin skin testing is strongly encouraged for participants working with non-fixed lung or lymph node tissue in Section X.

(B) A single tuberculin skin test is offered to participants working with all other nonhuman primate tissue. If the tuberculin skin test is negative and there are no other medical contraindications, the employee is medically cleared for work and there is no follow-up. If the tuberculin skin test is positive the course of action is as described in Section VII.

IX. Surveillance Recall

(A) Participants working with small and large animals are advised to return for tetanus (Td) boosting 10 years from the date of their last booster dose.

(B) Participants working with live nonhuman primates or non-fixed lungs or lymph nodes from nonhuman primates should return to Occupational Health Services as follows: If the prior tuberculin skin test was negative, the test is repeated every six months. If the prior tuberculin skin test was positive, regardless of whether chemoprophylaxis or treatment was received, the employee will be sent an informational TB health review annually with a letter asking the worker to call Occupational Health Services if he or she has any symptoms suggestive of active tuberculosis.

(C) Participants working with vaccinia are offered vaccinia vaccination and a booster every ten years

X. Summary
Requirements for certification of enrollment and continuing participation in the AESP:
(A) Participants working with small animals: Medical counseling (Section V.) and Tetanus immunization (Section V.)
(B) Participants working with large animals: Medical counseling (Section V.), Tetanus immunization (Section V.), Rabies immunization, if applicable (Section VI.) and Serologic testing for toxoplasmosis, if applicable (Section VI.),

(C) Participants working with live nonhuman primates: Medical counseling (Section V), Tuberculosis screening (Sections VII), Rubella immunization/protection (Section VII), Rabies immunization, if applicable (Section VI) and Bloodborne Pathogen Program (Section VII).

(D) Participants working with non-fixed tissues from nonhuman primates: Medical counseling (Section V) and baseline tuberculosis screening (Section VII).

XI. Surveillance Program Compliance

(A) A list of employees enrolled in the AESP is maintained by Occupational Health Services. Periodically, the Institutional Animal Care and Use Committee is informed of the individuals who have failed to participate in the AESP for disciplinary action.

(B) The AESP database contains the following information for each participant: name, last four digits of the social security number, category of animal used or cared for, date enrolled, immunizations, tuberculin testing and other surveillance examinations and dates of visits, immunizations and surveillance activities.

(C) Individuals who work with animals, but do not participate in the AESP may have their privileges to utilize laboratory animals suspended until successful completion.

Research Animal Resources

Sources of Research Animals

The majority of animals used in this institution are purchased from commercial suppliers and dealers. Commercial sources of rodents and rabbits are more numerous than those which supply dogs, cats, farm animals and nonhuman primates. Without prior arrangement with Dr. Robert Adams, no animal may enter any Johns Hopkins animal facility except through Research Animal Resources (Animal Resources) (410 955-3273).

Rodents

Rodents purchased from JHU-approved vendors and transported in vendor clean trucks may be brought directly into JHU rodent colonies, which are maintained free from a wide variety of rodent pathogens. Orders from approved vendors must be processed through the Research Animal Resources (RAR) procurement office at 410 955-3713.

Rodents acquired from non-approved vendors or other institutions must be brought in through rodent quarantine, where they are assessed for excluded pathogens. To initiate the import process, submit a completed "request to import rodents" form to the RAR import/export coordinator. This form can be downloaded from the Animal Order section of the RAR website. Although investigators do not have access to their rodents while they are in quarantine, RAR employs a skilled technician to provide a number of services in quarantine to mitigate research delays, including breeding, genotyping, sample collection, cross fostering and basic procedures. More information can be found on the RAR website.

JHU rodents to be exported to collaborators in the US or abroad are processed through the RAR import/export coordinator. To initiate the export process, submit a "request to export rodents" form.

A complete list of excluded pathogens and quarantine procedures, together with request forms can be found on the RAR website.

Poultry

Over the years we have had periodic problems with a herpesvirus which causes a neoplastic condition known as Marek's Disease. Marek's Disease is characterized by lesions in nervous and other tissues which cause weakness, paralysis and eventual death. Once the disease gets into a "flock" (or in our case a housing facility) it is spread through feather dander from infected to susceptible birds. It is a disease that affects birds less than 16 weeks of age. There is a vaccine available. Recommendations:

a. Investigators who use chicks and birds within one to two weeks of arrival do not need to worry about this
disease and do not need to purchase vaccinated chicks.
b. Investigators who purchase birds less than 6 weeks of age but intend to keep the birds for a long period of time should specify that they want vaccinated birds when they place their orders.
c. Investigators who use birds 6-7 weeks of age and intend to use them quickly probably do not need to order vaccinated birds.
d. Investigators intending to keep any bird for very long periods of time (i.e. for antibody production) should order vaccinated birds.
e. Find out from the vendor how long it will take to receive the order so the appropriate aged chicks arrive.

Rabbits
Rabbits are available from a number of different sources. Johns Hopkins University uses only Pasteurella free rabbits.

Cats
Johns Hopkins University uses only purpose bred cats.

Dogs
Johns Hopkins University uses only Class A dogs. Dogs are delivered directly to the Miller Research Building loading dock. They are processed and identified with an implanted electronic chip.

Class A: Commercially raised purebred or mongrel dogs: Beagles and hound-type dogs are the usual breeds raised commercially. These animals are of excellent quality and uniformity.

Farm Animals
Farm animals required for research are obtained from a commercial supplier. Special requests, such as pregnant sheep or goats, must be placed well in advance. Small ruminants are seasonal breeders and obtaining pregnant animals in late spring, summer or fall is difficult.

Nonhuman Primates
All nonhuman primates should be considered possible potential carriers of biohazardous agents (Tuberculosis, Herpesvirus B, Entamoeba histolytica, etc). Investigators should be aware of all possible hazards and pass such information on to their research personnel. Masks, gloves, eye and mucus membrane protection and protective clothing are needed when working with these animals. Please contact Research Animal Resources for specific guidelines and instruction.

All primates from outside sources must undergo a quarantine period usually lasting a minimum of 6 weeks. Quarantine space is allocated on a first come, first served basis. Many species are available from commercial importers and breeders. Some species are not obtainable from the wild because of restrictions imposed by the country of origin.

A small number of rhesus and pigtail monkeys are raised within this institution. They are usually available in weights up to 2-3 kg. Adult reproductive culls are available periodically as well. Some species (rhesus, cynomolgus, squirrel) are available from NIH supported colonies for investigators having NIH funds. In the case of rhesus monkeys, if the desired animal is available in our institutional colony it will come from that colony preferentially. When excess animals are available within this institution, a memo is sent to all primate users advising them of the availability. If you wish to be put on this list, or if you wish to make animals available, please call Dr. Adams at 410-955-3273.

Primate Clearing House: At the national level excess animals are advertised weekly in a publication from the University of Washington, Primate Research Center. Many species are often available. Investigators can also advertise for desired tissues, organs, etc.

Animal Purchasing
All animals are purchased through SAP. The approved order must be received by Animal Resources by Wednesday to receive animals the following week.

All animal-based research must be approved by the Institutional Animal Care and Use Committee. To assure this,
all investigators will be asked to provide the identification number of the approved protocol (protocol number) at the
time they are placing orders for animals. Protocol numbers are species coded; investigators must make sure that
the protocol number used is correct for the procedure and species to be used. The number of animals purchased
will be debited from the approved number. Once the approved number is reached, the protocol must be amended
to request additional animals.

Animals are delivered to Johns Hopkins at a central receiving dock. After arriving, animals are checked for
accuracy and condition and then sent out by Research Animal Resources to the proper animal facility for housing.
Any animals arriving without an Animal Resources purchase order number will not be delivered. Occasionally an
investigator has animals coming from private sources or the animals are purchased through another institution and
sent to Hopkins. We can arrange delivery of these animals only if the investigator notifies us in advance of the
expected shipment. It is important that we be notified of such animals for two reasons: to have appropriate caging
available; and to guard against infections being introduced into Johns Hopkins’s facilities which could jeopardize
other investigators’ animals.

**Charge Authorization and Billing**

Investigators must obtain an Animal Resources job number. This authorizes Animal Resources to automatically
transfer funds from a university budget to cover the cost of animal care and services. Investigators will be asked to
supply us with an account number and expiration date and the name and address of the individual to receive
statements. Current research protocols must be on file before an account can be opened.

Charge labels for each Animal Resources job number will be supplied. These labels should be affixed to the
census sheet before it is sent to the Animal Resources office for billing. This indicates your agreement with the
information on the census. Census sheets will remain in the animal area for three days following the end of each
month to allow for verification and change. It is difficult to correct errors once a bill has been processed because
considerable time has elapsed and documentation is usually difficult to obtain. Additional labels will be printed as
needed; call Animal Resources at 410-955-3713.

Investigators will be sent reminders several months prior to the job number’s expiration. This will allow sufficient
time to supply us with a new budget number before the account expires.

If you have questions about charge authorizations and billing, contact Animal Resources at 410-955-3713.

**Animal Housing and Costs**

The following areas are used for the housing of research animals. All species cannot be housed in every area. The
housing areas are: Pathology (primate only), Blalock, Woods Research Building, Cancer Research Building I and II,
Miller Research Building, Ross Research Building, Bloomberg School of Public Health, Bayview Medical Center,
Asthma and Allergy Center, Homewood Campus, School of Nursing and Johns Hopkins University Research Farm.

Animals will be housed as close as possible to investigators, dependent upon available space, NIH requirements
regarding species separation, and needs of other investigators. *Per diem* charges are assessed for each animal to
cover feeding, watering, bedding, sanitation, transportation, equipment maintenance, etc., in accordance with the
NIH Rate-Setting Manual. Contact Research Animal Resources (410-955-3713) for the current daily per diem
charges per species.

The animal census is kept on a census sheet in each housing area. These census sheets are collected three days
after the last day of the month, and charges are calculated for each investigator’s budget. Animal Resources care
staff will add incoming animals and subtract those that are found dead. It is each investigator’s responsibility to
subtract animals used for experimental purposes. Animals born in the facility are added to the census at weaning.
A sample census sheet is found at the end of this book (forms).

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**Veterinary Medical and Ancillary Services**

In accordance with the generally-accepted tenet that comfortable, healthy, genetically and nutritionally appropriate
animals kept under optimal environmental conditions are more likely to yield fruitful research results and in compliance with the various laws and accreditations under which Johns Hopkins operates, a complete spectrum of veterinary medical services is available. Many of these services are available without cost to the scientist.

Technical Services
Research Animal Resources will provide technical services such as blood withdrawal, administration of anesthetics, animal transportation, etc. to Hopkins investigators in support of their research activities. Charges are incurred for these services. By advance consultation, budgeting for these services can be included in research grant applications. For rodents, contact Dr. Julie Watson at 443-287-2953. For all other species, contact the RAR technicians at 410-502-5063.

Clinical Medicine
When an investigator, research staff member, fellow, student, technician or any other person associated with institutional animal use believes an animal is abnormal, sick, in discomfort, or otherwise requiring aid, a call should be placed to Animal Resources at 410-955-3273. A staff veterinarian will respond and take appropriate action, in consultation with the investigator. It is essential that clinical calls be initiated by the investigator, student, fellow or technician at the earliest sign of any abnormality. Complete diagnostic laboratories, surgical and radiographic facilities, and consultative services are also available by calling 410-955-3273.

After regular working hours and/or weekends or holidays, the veterinarian on call can be reached by beeper at 410-283-0929 or you can leave a message on Animal Resources voice mail at 410-955-3273. Voice mail messages will be answered the following business day.

Post-procedural care is the responsibility of the principal investigator and associated staff. The veterinary staff is available for consultation and advice if problems arise.

Veterinary rounds are periodically made through each animal holding facility. During these rounds veterinarians from RAR are looking for husbandry and animal health problems. If any are noted, the investigator is contacted and a plan of action is determined after consultation.

Pathology
Through the comparative pathology section, the Department provides diagnostic pathology service to investigators at no charge using animals in their research. The purpose of this service is to identify intercurrent disease in the animal population. Pathologic examination will be performed on animals dying unexpectedly and not as a result of experimental procedures. Animals and tissues should be submitted to the necropsy room on the ground floor of the Traylor Building as soon as possible after death or for sacrifice; carcasses should not be frozen. A complete description of the animal's history should be included. A preliminary diagnosis will be rendered following the gross examination. Final diagnosis including histopathology, microbiology, and other diagnostic procedures will follow as soon as possible. Arrangements for pathology can be made through 410-955-3273.

Gross and histopathology for experimental protocols is not provided except by prior arrangement with members of the Department for scientific collaboration in the usual manner. For more information about collaborations, call 443-287-2953.

Support Services
Surgery Support:
The veterinarians in Research Animal Resources are available to train or perform various surgical procedures. For more information, call 410-955-3273. In addition, the veterinary technical staff is available on a fee-for-service basis to provide support for surgical procedures performed in the Research Animal Resources surgical facilities in the Ross Research Building. These trained personnel can be of great help in experimental surgical procedures and other manipulations of laboratory animals. These technical services can be obtained through prior arrangement by calling 410-502-5063.

Histology Laboratory
This laboratory processes specimens for light microscopy. Most cases are presented as tissues fixed in formalin which are to be embedded in paraffin for histologic slide preparation. A wide variety of special stains are available.
by request. Special guidance with tissue trimming, procedures for serial sectioning, and preparation of sectioned specimens for other types of procedures, such as immunohistochemistry, autoradiography, etc. may be made available by special arrangement. Contact 410-955-8728 for additional information.

Clinical Pathology Laboratory
The Department's clinical pathology laboratory has considerable experience with hematology, serum chemistry, urinalysis and fecal parasite examinations in a wide variety of species. These examinations are available on a fee-for-service basis to support experimental protocols. Specimens for clinical chemistry or other specialized analysis can be handled through this laboratory. Diagnostic bacteriologic techniques focused specifically on animal pathogens can also be arranged through this laboratory. For details, call 410-955-3273.

The rodent Phenotyping Core can also provide hematology and serum chemistry analysis. Call 410-502-3050 for additional information.

Pre-Research Consultation
The Department, through its faculty and its specialized departmental library, has available information and advice regarding:

- Selection of appropriate animal species to carry out specific studies, or non-animal techniques as they are published.
- Existing animal models of human diseases.
- Anatomical and physiological peculiarities of most animals used in research.
- Techniques of anesthesia, analgesia and chemical restraint, and dosages.
- Techniques of blood and other sampling and drug or chemical administration.
- Pathological and clinical effects of intercurrent animal disease.
- Special caging or experimental techniques.

We encourage such consultations prior to the preparation of grant and contract applications. Call 410-955-3273 to arrange a consultation.

Survival Surgical Procedures
The Institutional Animal Care and Use Committee has set minimum standards for animal operating rooms and laboratories in which surgery is performed. The standards are based on the interpretation by the Institutional Animal Care Committee of the NIH Guide for the Care and Use of Laboratory Animals. Adherence to these guidelines is necessary in order for us to comply with the standards of our accreditation agency, the Association for the Assessment and Accreditation of Laboratory Animal Care International. The standards are meant to ensure that surgical procedures are performed in an appropriate environment using good surgical techniques.

Aseptic technique should be used for surgical procedures on rodents such as rats and mice; however, the standards for the surgical facility are different. Rodent surgical areas, which may either be a separate room or portion of a room, should be clean and orderly and not used for any other purpose during the time of the surgical activity.

For all other species, designated survival surgical areas should be used only for that purpose. Use of the area for other purposes such as office space and equipment and supply storage, except for surgical and research equipment, is not acceptable. Built-in cabinets and sinks should not be placed in the operating room.

For purposes of definition, non-survival or acute surgical procedures are those in which the animal is euthanized at the termination of the procedure. The animal is never allowed to regain consciousness or the ability to perceive pain. Survival surgical procedures are those from which the animal is allowed to regain consciousness and survive for some period of time. Survival surgical procedures must be done using aseptic techniques, in areas approved for such purposes.

Non-survival surgical procedures may be performed in general purpose laboratories under the following conditions: a) there must be no eating or drinking in the laboratory during the surgical procedure; b) all equipment and surfaces within the room must be kept clean; and c) the surgical area should be free of non-essential equipment and
supplies.

Other guidelines:

- Personnel performing surgery should have formal training in operative procedures and aseptic technique or have acceptable career experience.
- Anesthetic techniques and use of pre-emptive and post operative analgesia should be in accordance with current methods in the literature and should be recorded.
- The investigator is responsible for post operative monitoring of animals. Animals should be closely monitored during recovery from anesthesia in a recovery cage until they have regained consciousness and can maintain sternal recumbency. This cage should be kept in the designated prep/recovery area or a separate recovery room if it is available. After recovery from anesthesia animals should continue to be monitored for pain, discomfort or other problems until the incision is healed. A daily log of treatments and the animal’s status is required. Records must be available to the veterinary staff, Institutional Animal Care and Use Committee, and United States Department of Agriculture inspectors and other relevant federal or funding agency for review. A suggested format for these records is included in the Forms section.
- Multiple survival surgery procedures on animals for economic purposes alone are not acceptable. Multiple surgical procedures may be performed on the same animal if they are related components of a project and have been reviewed and approved by the Institutional Animal Care and Use Committee.
- Proper methods of euthanasia should be used at the termination of the experiment and should be documented. Another source of information is the AVMA Guidelines on Euthanasia.
- Animal Resources has survival surgical areas located in Ross Research Building and Asthma and Allergy Center. Contact Research Animal Resources for assistance in gaining access to these areas. Surgical equipment such as appropriate instrument packs, monitoring equipment, and life support equipment should be available. To use approved survival surgery areas controlled by individual departments or investigators please contact them directly.
- If explosive anesthetic agents such as ether are used, explosion proof electrical outlets or outlets five feet above the floor are necessary. Use of these agents in explosion proof hoods is also acceptable. The Safety Office strongly discourages the use of ether because of its explosive potential. If volatile anesthetic gases are used, a gas evacuation and scavenging system is necessary. Eating and drinking are not allowed in the operating suites.

Animal Surgical Suites

Introduction
The Animal Welfare Act and the Public Health Services Policy (as stated in the Guide) require use of aseptic procedures for survival surgical procedures. Aseptic technique includes preparation of the animal such as hair removal and disinfection of the operative site; preparation of the surgeon such as provision of decontaminated surgical attire, surgical scrub and sterile gloves; sterilization of instruments, supplies and implanted materials; and use of operative techniques to reduce the likelihood of infection. Major operative procedures on non-rodents will be conducted only in facilities intended for that purpose (dedicated surgical facility), which are maintained for use under aseptic conditions. Non-major operative procedures and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic procedures. Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures. The following criteria should be met for such a facility:

Operating Room
1. The floor, ceiling, and walls must be created by a continuous connection, constructed of materials that are easily sanitizable and must be kept physically clean. Interior surfaces should be constructed of materials that are monolithic and impervious to moisture.
2. Supplies and equipment not relevant to the surgical procedures being performed should not be stored in the room. The operating room cannot be used as an office, laboratory or storage room.

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1 Approved by the IACUC on: 1/17/2002
2 9 CFR Chapter 1 Subchapter A, Parts 1,2 and 3
3 Guide for the Care and Use of Laboratory Animals (8th ed.), NRC, National Academy Press, 2011

Revised 1/6/09, updated 2/27/13, 10/20/15
3. A surgical light and an easily sanitizable surgical table must be available.

4. Appropriate scavenging must be provided whenever gas anesthesia is used.

5. The operating room must normally be used only for aseptic surgery. A non-aseptic surgery may be performed, however, provided the operating room is thoroughly decontaminated prior to performing the next aseptic surgical procedure. Decontamination consists of cleaning the ceiling, walls, floors and equipment with a disinfectant. A record of decontamination must be maintained.

Surgical Support Rooms

1. There must be at least two surgical support rooms separate from the operating room, one for surgeon’s preparation and the other for animal preparation. The former may also be used for instrument and pack preparation and the latter for post-operative recovery.

2. Surgeon Preparation Room – The surgeon should scrub (prepare) in a room separate from both the animal preparation room and the operating room. This is also where the surgeon dons a facemask and head and shoe covers. The surgeon preparation room should be contiguous with the operating room. Instrument cleaning and pack preparation may be done in this area but must not occur in the operating room. If the surgeon preparation room is used for other activities as well, all other activities must cease prior to and during the surgeon’s scrub so that aseptic preparation of the surgeon is not compromised.

3. Animal Preparation Room – Preparation of the animal (i.e. inducing anesthesia, clipping and preliminary surgical scrub) must be performed in a room separate from the surgeon preparation room and the operating room. The animal preparation room need not be contiguous with the operating room. After the animal has been moved to the operating room, a final scrub should be performed on the operating table.

The animal preparation room may also be used for intensive care and support treatment during the post-anesthetic recovery period. Only uncomplicated and short anesthetic recovery (less than one hour to sternal or sitting position) can occur in the animal’s home cage. Postsurgical (post anesthetic) monitoring and record keeping must be in accordance with current rules, regulations and standards.

A well-designed and operated surgical suite should have the following additional features:

1. No recirculation of room air, unless particulate or noxious gaseous contaminants have been removed.
2. Air supply and exhaust plenum should not be located over the surgery table.
3. Operating room should be at positive pressure relative to the adjacent preparation areas or hallways to prevent dust contamination of the room.
4. Lockers and an area for dressing into surgical attire are desirable.
5. Convenient access to an autoclave and gas sterilization equipment.
6. Oxygen and suction should be available.

Assessment of Pain in Laboratory Animals

In order to detect signs of pain, it is critical that you familiarize yourself with an animal’s normal and abnormal behavioral repertoire. Veterinarians and animal care technicians are uniquely trained to identify signs of pain and distress in animals. For assistance or questions about pain and distress, please contact Research Animal Resources (410-955-3273). Signs of pain and distress vary between species and within individual animals and may be further modified by previous experience of the animal (e.g. surgery, drugs, stress, health status). It is important to remember that signs may not be present all the time. Pain intensity and/or responsiveness to pain may also vary in a given animal.

Always consult a clinical veterinarian for advice on care of animals and especially after observing signs as described below regardless of whether or not a procedure has been performed on the animal. It is critical that you consult with a veterinarian during the planning of your animal use protocol so that appropriate criteria for monitoring, intervention strategies and endpoints can be determined.

<table>
<thead>
<tr>
<th>Common indicators of pain</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeanor/Attitude</td>
<td>Quiet, depressed, apprehensive, fearful, anxious, restless, irritable</td>
</tr>
<tr>
<td>Behavior</td>
<td>Reduction in activity, interaction, exploratory behavior, grooming, food and water intake</td>
</tr>
<tr>
<td>Posture</td>
<td>Crouched, hunched, abnormal lying position, favoring a painful limb, head banging, head pressing</td>
</tr>
<tr>
<td>Region</td>
<td>Signs of pain</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Head, ear, throat, mouth</td>
<td>Rubbing, shaking, head tilt, head pressing or holding, “stiff-neck posture”, self-mutilation, depression, and reluctance to swallow, eat, drink or move</td>
</tr>
<tr>
<td>Ophthalmic</td>
<td>Reluctance to move, scratching, rubbing, squinting (blepharospasm), discharge, redness</td>
</tr>
<tr>
<td>Orthopedic</td>
<td>Abnormal posture, abnormal gait, reluctance to move, guarding, licking, biting, self-mutilation</td>
</tr>
<tr>
<td>Abdominal</td>
<td>Protecting painful area (guarding), abnormal posture, unwillingness to stand, licking or looking at area, vomiting, anorexia</td>
</tr>
<tr>
<td>Thoracic</td>
<td>Change in respiratory rate and pattern (abdominal breathing, short shallow breaths), reluctance to move, pain on chest compression, anxiety, cyanosis (blue)</td>
</tr>
<tr>
<td>Perineal</td>
<td>Scooting, biting, licking, self-mutilation</td>
</tr>
</tbody>
</table>

### Signs associated with acute pain

<table>
<thead>
<tr>
<th>Signs associated with acute pain</th>
<th>Signs associated with chronic pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased food and water intake</td>
<td>Decreased appetite</td>
</tr>
<tr>
<td>Acute weight loss</td>
<td>Chronic weight loss, poor body condition</td>
</tr>
<tr>
<td>Protecting (guarding) painful area, teeth grinding in some species</td>
<td>Alterations in urinary and bowel activities</td>
</tr>
<tr>
<td>Vocalizing, especially on palpating painful area or following movement</td>
<td>Behavioral changes e.g. aggression, withdrawn, hypersensitive, unresponsive</td>
</tr>
<tr>
<td>Licking, biting, scratching, or shaking affected area, flinch</td>
<td>Self-mutilation</td>
</tr>
<tr>
<td>Restlessness (e.g., pacing, repeated lying down and getting up)</td>
<td>Increased tear production, porphyrin staining around eyes (particularly in rats)</td>
</tr>
<tr>
<td>Lack of or reduced mobility, lethargic</td>
<td>Reduced activity, lethargic</td>
</tr>
<tr>
<td>Failure to groom, ruffled fur</td>
<td>Lack of grooming</td>
</tr>
<tr>
<td>Abnormal posture, hunched up</td>
<td>Decreased litter size</td>
</tr>
<tr>
<td>Separation from group, hiding</td>
<td>Separation from group</td>
</tr>
<tr>
<td>Depression, lack of inquisitiveness</td>
<td></td>
</tr>
</tbody>
</table>

### Signs of Pain in different species

**Nonhuman primates**

<table>
<thead>
<tr>
<th>Signs of pain</th>
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</thead>
<tbody>
<tr>
<td>Behavior</td>
</tr>
<tr>
<td>Attitude</td>
</tr>
<tr>
<td>Clinical signs</td>
</tr>
<tr>
<td>Dogs</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Quiet, inattentive, recumbent, apprehensive, aggressive</td>
</tr>
<tr>
<td>Flinch, stiff, reluctant to move, abnormal posture, attempts to escape</td>
</tr>
<tr>
<td>Bite, scratch, shake or guard painful site</td>
</tr>
<tr>
<td>Lack of appetite, fast breathing, panting</td>
</tr>
<tr>
<td>Whimper, howl, growl</td>
</tr>
<tr>
<td>Facial expression may/may not change</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rabbits Signs of pain</th>
<th>Guinea pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavior Inactive, hunched, hiding, withdrawn, aggressive, vocalize, increased activity, licking, scratching or biting affected site, self-mutilation</td>
<td></td>
</tr>
<tr>
<td>Attitude Apprehensive, anxious</td>
<td></td>
</tr>
<tr>
<td>Clinical signs Increased respiration, lack of appetite, immobility, salivation, teeth grinding, struggling on handling, fever, poor fecal production</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rats and Mice Behavioral changes, struggling, biting, vocalization, depression, non responsive</th>
<th>Vocalization, attempts to escape, stampede, reluctance to move, altered gait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor body condition, wasting, dehydration, decreased growth and reproduction, sunken eyes, eyelids closed</td>
<td>Timid, apprehensive, passive, unresponsive</td>
</tr>
<tr>
<td>Fast and labored breathing, shallow infrequent respiration, chattering (mice), sneezing (rats), porphyrin staining around eyes and nose, pale or bluish extremities</td>
<td>Cold, bluish extremities</td>
</tr>
<tr>
<td>Pilorection (spiked hair coat), decreased grooming, dirty hair coat, scratching</td>
<td></td>
</tr>
<tr>
<td>Hunched, crouched, recumbent</td>
<td></td>
</tr>
<tr>
<td>Decreased exploration, lethargic, isolation from cage mates, cold</td>
<td></td>
</tr>
<tr>
<td>Lack of appetite, no feces, pica, cannibalism</td>
<td></td>
</tr>
<tr>
<td>Excessive licking, scratching, self-mutilation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sheep and Goats Decreased appetite, decreased rumen sounds, depressed, dullness, increased respiration, grunting, lameness, tearing, straining, agitation, resent handling, aversion to handler, reduced exploratory behavior</th>
<th>Pigs Reluctance to stand or move, hiding, separation, loss of appetite, pilorection (hair stands up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in posture, repeated lying down and standing up, reluctance to move, separation for others</td>
<td>Squealing on palpation of painful area, changes in vocalization</td>
</tr>
<tr>
<td>Teeth grinding, apprehension, decreased response, head carried low, ears down or flat, separation</td>
<td>Aggression</td>
</tr>
<tr>
<td>Vocalization Changes in gait, posture, respiration, temperature, change in skin color</td>
<td></td>
</tr>
</tbody>
</table>
Pain Scoring

Pain scoring systems have been developed to quantify the degree of pain experienced by an animal. For such a system to be successful it must be easy to use, acceptable to all parties, adhered to by everyone in the laboratory, tailored to the specific procedure(s) and reproducible. Scoring systems often incorporate behavioral variables and clinical signs, and action to be undertaken depending on the score. Since many pain score scales are initially developed with minimal data from the study, the systems must be flexible and allow for review and adjustment in values (scores) assigned to each parameter in future studies after sufficient data has been collected.

References

13. Liles JH, Flecknell PA. The effects of buprenorphine, nalbuphine and butorphanol alone or following halothane anaesthesia on food and water consumption and locomotor movement in rats. Laboratory Animals 26: 180-189. 1992


### Procedures in Common Laboratory Rodents and Rabbits

#### Handling Mice

**Identification**
All animals should be properly identified. Identification systems include cage cards, ear tags and notches, tattoos, transponders, fur dyes, etc. Cage cards should include source of the animal, strain or stock, names and contact information of responsible investigators, pertinent dates and protocol number. The chart shows an example of an ear notching system.

**Handling**
Mice should be gently acclimatized to handling to reduce stress. Always talk quietly, move hands slowly and handle the mice frequently. Mice should be handled at the base of the tail using your fingers or with forceps. Transfer the mice to a firm surface and apply a **scruff hold** to the loose skin between the ears with your thumb and forefingers while maintaining a grip on the tail. Do not pull the skin too tightly as the mice can choke. Too loose of a hold will allow the mouse to turn its head and bite. This hold allows you to examine the under belly and perform other procedures. A variety of restraint devices are available to assist in handling mice. The [Animal Care and Use Committee](#) offers periodic rodent handling courses.

#### Blood Collection in Mice

An adult mouse has a circulating blood volume of about 1.5-2.5 ml (6-8% of the body weight) however in older and obese animals this value may be lower. Up to 15% of the circulating blood volume (1% of normal body weight) may be taken on a single occasion from a normal healthy animal on an adequate plane of nutrition with minimal adverse effect. Always make sure the animal has recovered safely from the procedure and give warm isotonic fluids. This volume may be repeated after 4 weeks. For repeat bleeds at shorter intervals, please see the [Guidelines for Multiple Blood Draws](#). When withdrawing large volumes of blood, additional monitoring of hematological parameters, animal condition and appropriate fluid replacement should be performed.

Below is a table showing blood sampling volumes and recovery periods:

<table>
<thead>
<tr>
<th>Multiple sampling</th>
<th>Approximate Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating blood volume removed in 24 hours</td>
<td>1 weeks</td>
</tr>
</tbody>
</table>
Blood can be collected from several sites in the mouse including facial vein, tail vein, saphenous vein, retro-orbital sinus, brachial vessels, vena cava or cardiac puncture. For tail vein and saphenous blood collection, the area should be cleaned with alcohol. This will also provide better visualization of the vessel. Some procedures will require anesthesia; others may be carried out without anesthesia, provided suitable restraint is possible. In order to visualize veins better, one of several methods of dilation may be used. The vessel may be occluded and pressure will cause some enlargement. Heat will also cause dilation. Always ensure complete hemostasis before returning the mouse to its home cage.

**Facial vein**

Small quantities of blood can be obtained in an unanesthetized mouse via the facial vein. Scruff the mouse just so the eyes slightly bulge and the mouth is open. Locate the small hairless area on the side of the jaw. Use a lancet or an 18-20 gauge needle to prick the skin next to the hairless area. Advance the needle only to the top of the bevel. Have your collection tube ready. As soon as the needle is removed, you may be able to collect 4-7 drops of blood.

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**Tail**

It may be necessary to warm the tail by exposing it briefly to a heat lamp or placing it in a bowl of warm water. Be very careful when using heat lamps because they can cause thermal burns. The mouse should be restrained in a device for the collection. Blood can be collected from the tail vein (and artery) by making a snip in the terminal ≤ 3 mm of the tail with a scalpel or sharp scissors. Stroke the tail gently with thumb and finger to enhance flow of blood into the collection vial. Because of the thermoregulatory

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Photo courtesy of: [http://www.ahc.umn.edu/rar/facial_vein.htm](http://www.ahc.umn.edu/rar/facial_vein.htm)
function of the tail, no more than the distal 3 mm should be taken at a time. At the end of the collection apply pressure to the cut end with a gauze bandage and ensure that blood has completely stopped flowing before returning the mouse to the cage. A small nick can also be made at side of the tail 0.5–2 cm from the tail base to collect blood. A fine gauge needle introduced through the skin at a shallow angle can be used to withdraw blood from the tail vein. Apply a tourniquet around the base of the tail to aid in the collection. A butterfly catheter with only about 5 mm of tubing attached to it (rest cut off) may be used instead of a needle and syringe.

Saphenous vein
Restrain and extend the hind leg applying gentle downward pressure above the knee joint on the lateral aspect. This stretches the skin making it easier to shave and immobilizes the saphenous vein. Wipe the shaved area with alcohol and use a 25-gauge needle to puncture the vein. If done correctly a drop of blood forms immediately at the puncture site and can be collected in a microhematocrit tube. Gentle pressure over the puncture site or relaxation of the restrainer’s grip is usually sufficient to stop the blood flow. The scab at the puncture site can be removed at a later date to allow additional blood collection.

Blood collection using the saphenous vein.

Retrorbital sinus
The retroorbital sinus is a system of dilated venous channels at the back of the orbit. Blood can be collected from this area in anesthetized mice using a microhematocrit tube. There should be no movement of the head during the procedure. Typically anesthesia is required unless the lab member is very experienced in this blood collection technique. Pressure down with the thumb and forefinger just behind the eye and pull back on the skin to allow the eyeball to protrude. Position a microhematocrit tube along the inner corner of the eye (medial canthus) beside the eyeball. Insert the tube gently but firmly through the conjunctiva towards the back of the eye along the orbit. Rotate the tube gently as you proceed. Blood should flow freely if the tube is properly inserted. Tilt the head slightly downward to improve flow. After collecting the blood withdraw the tube and apply pressure on the closed eyelids to stop any bleeding. Remove excess blood with gauze. Complications include damage to the eye and surrounding tissues.
Brachial vessels
Blood can be collected from the brachial plexus as a terminal procedure in deeply anesthetized mice. Make a cut through the skin at the side of the thorax into the angle of the forelimb (axilla) to expose the axillary vessels. Transect the vessels and allow blood to pool into the pocket created by tenting the skin. Aspirate the mixed venous arterial blood into an appropriate receptacle.

Vena cava and abdominal aorta
Blood can be obtained from the posterior vena cava or abdominal aorta in a deeply anesthetized mouse following laparotomy. Approach the vessel at a shallow angle using a fine gauge needle attached to a small syringe. This is a terminal procedure.

Cardiac puncture
Up to 1 ml of blood can be obtained from the heart of a deeply anesthetized mouse in a terminal procedure. The most common approach is to lay the mouse on its back and insert a 25 to 30 gauge needle attached to a 1ml syringe just behind the xiphoid cartilage and slightly left of the middle. The needle should be introduced at 10-30 degrees from the horizontal axis of the sternum in order to enter the heart. Alternatively approach the heart laterally immediately behind the elbow at the point of maximum heartbeat.

Administration of Substances in Mice
Materials to be administered to mice can be given orally (e.g., in water or feed) or injected systemically through a variety of routes. The average daily consumption of feed and water for an adult 25 g mouse is 3-5 g and 4 ml respectively. The following volumes can be injected into mice safely (based on 25 g mouse): 2-3 ml subcutaneously, 0.05-0.1 ml intramuscularly (0.03 ml per site), 0.5 ml intravenously, 0.1-0.3 ml into the stomach and 2-3 ml intraperitoneally. Intramuscular injections are usually not recommended in mice because of their small muscle mass. If an intramuscular injection is performed, a fine gauge needle should be used and the injection into the cranial thigh muscle.

Gavage
Oral gavage is performed using a ball ended feeding tube. Estimate the distance that the tube needs to be inserted into the mouse (usually from the nose to the first rib) and mark it on the tube. Restrain the mouse with the head and body extended as straight as possible to facilitate introduction of the gavage tube. Introduce the tube in the space between the left incisors and molars, and gently direct it caudally toward the right ramus of the mandible. The mouse usually swallows as the feeding tube approaches the pharynx, facilitating entry into the esophagus. If the animal struggles or appears to be in respiratory distress withdraw the tube and begin all over again.
Once the desired position is attained, slowly inject the material and withdraw the tube. Monitor the animal after the procedure to ensure that there are no adverse effects.

Subcutaneous injections:
Subcutaneous injections are usually made into the loose skin over the neck or flank using a fine gauge needle. Grasp the skin. Insert the needle 5-10 mm through the tent in the skin before making the injection. Lack of resistance to the injection is indicative that you are in the right location. Check for leaking on the other side of the skin especially if a larger volume is injected.

Intraperitoneal injections
Intraperitoneal injections are usually made in the lower right quadrant of the abdomen. The mouse is restrained with its head tilted lower than the body to avoid injury to internal organs. After swabbing the lower right quadrant with alcohol, a fine gauge needle is introduced slowly through the skin, subcutaneous tissue and abdominal wall. Withdraw the syringe plunger to ensure that you are not in the bladder or intestines. If nothing is withdrawn inject the material slowly. If you accidentally enter the bladder or intestines withdraw and discard the needle and syringe.

Intravenous injections
Intravenous injections are usually made into the dorsal tail vein. Warm the tail by immersing it in warm water or placing the animal under a heat lamp. This may help dilate the vessel and make it easier to visualize. The tail vein is easier to see in non-pigmented mice. A fine gauge needle should be used for this procedure.

Normative Values in Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifespan</td>
<td>1-3 years</td>
</tr>
<tr>
<td>Adult weight</td>
<td>Males 20-30 g, females 18-35 g</td>
</tr>
<tr>
<td>Birth weight</td>
<td>1-2 g</td>
</tr>
<tr>
<td>Heart rate</td>
<td>310-840 beats per minute</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>80-230 breaths per minute</td>
</tr>
<tr>
<td>Body temperature</td>
<td>36.5°-38°C</td>
</tr>
<tr>
<td>Blood volume</td>
<td>7-8%, 1.5-2.5 ml</td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.5-1 ml per day</td>
</tr>
</tbody>
</table>

Revised 1/6/09, updated 2/27/13, 10/20/15
**Signs of Pain in Mice**

<table>
<thead>
<tr>
<th>Acute pain</th>
<th>Chronic pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia (no fecal pellets)</td>
<td>Decreased body weight</td>
</tr>
<tr>
<td>Decrease in appetite (few</td>
<td>Reluctance to move</td>
</tr>
<tr>
<td>fecal pellets)</td>
<td>Change in behavior</td>
</tr>
<tr>
<td>Rubbing or scratching surgical site</td>
<td>Poor grooming</td>
</tr>
<tr>
<td>Biting or shaking affected body part</td>
<td>Change in bowel or urinary activity</td>
</tr>
<tr>
<td>Restlessness</td>
<td>Rough hair coat</td>
</tr>
<tr>
<td>Porphyrin discharge (red-brown pigment around eyes and nostrils)</td>
<td></td>
</tr>
<tr>
<td>Increased respiration</td>
<td></td>
</tr>
</tbody>
</table>

Rodents in pain appear hunched, having a scruffy coat, lack a normal response to stimulus, tend to get cold and dehydrated and tend not to eat.

**Handling Rats**

Rats should be acclimatized to gentle handling to reduce stress. Always talk quietly, move hands slowly and handle them frequently. Rats should be initially picked up out of the cage by base of the tail using your fingers. Once out of the cage, the rat can be picked up by placing a hand firmly over the back and the rib cage and restraining the head with your index and second fingers immediately behind the mandibles. The presence of Chassaignac’s tubercle, a thin plate of bone extending from the transverse processes of the vertebrae make it difficult to grasp the skin of the back (scruffing). Holding the rat upside down keeps it distracted and reduces the chances of biting. Certain strains are more aggressive e.g. Fisher 344 is more aggressive than Sprague-Dawley, so care and experience are essential to rapid and safe handling. A variety of restraint devices are available to assist in handling rats.

**Blood Collection in Rats**

An adult rat has a circulating blood volume of about 15-35 ml (5-7% of the body weight), however in older and obese animals this value may be lower. Up to 15% of the circulating blood volume (or 1% of normal body weight) may be taken on a single occasion from a normal healthy animal on an adequate plane of nutrition with minimal adverse effect. Always make sure the animal has recovered safely from the procedure and give warm isotonic fluids. This volume may be repeated after 3-4 weeks. For repeat bleeds at shorter intervals, please see the [Guidelines for Multiple Blood Draws](#). When withdrawing large volumes of blood, additional monitoring of
hematological parameters, animal condition and appropriate fluid replacement should be performed.

Below is a table showing blood sampling volumes and recovery periods:

<table>
<thead>
<tr>
<th>Circulating blood volume removed in 24 hours</th>
<th>Approximate Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75%</td>
<td>1 weeks</td>
</tr>
<tr>
<td>10%</td>
<td>2 weeks</td>
</tr>
<tr>
<td>15%</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

Blood can be collected from several sites in the rat including the tail vein, saphenous vein, retro-orbital sinus, brachial vessels, vena cava or cardiac puncture. When venipuncture is required, hair should be shaved or at least clipped from the site for better visibility. The area of injection or incision should be cleaned with alcohol. Some procedures will require anesthesia; others may be carried out without anesthesia, provided suitable restraint is possible. In order to visualize veins better, one of several methods of dilation may be used such as occluding the vessel with a tourniquet so pressure can result in vessel dilation or warm the tail. Always ensure complete hemostasis before returning the rat to its home cage.

**Tail**

It may be necessary to warm the tail by exposing it briefly to a heat lamp or placing it in a bowl of warm water. Be very careful when using heat lamps since they can cause thermal burns. The rat should be restrained in a device for the collection. Blood can be collected from the tail vein (and artery) by snipping the terminal 5 mm of the tail with a scalpel or sharp scissors. Stroke the tail gently with your thumb and finger to enhance the flow of blood into the collection vial. At the end of the collection apply pressure to the cut end with a gauze bandage and ensure that blood has completely stopped flowing before returning the rat to the cage. A small nick can also be made at the side of the tail 1 – 3 cm from the tail base to collect blood. A fine gauge needle introduced through the skin at a shallow angle can be used to withdraw blood from the tail vein. Apply a tourniquet around the base of the tail to aid in the collection. A butterfly catheter with only about 5 mm of tubing attached to it (rest cut off) may be used instead of a needle and syringe.

**Saphenous vein**

Restrain and extend the hind leg applying gentle downward pressure above the knee joint on the lateral side. This stretches the skin making it easier to shave and immobilizes the saphenous vein. Wipe the shaved area with alcohol and use a 25-gauge needle to puncture the vein. If done correctly a drop of blood forms immediately at the puncture site and can be collected in a microhematocrit tube. Gentle pressure over the puncture site or relaxation of the restrainer’s grip is usually sufficient to stop the blood flow. The scab at the puncture site can be rubbed off at a later date to allow additional blood collection.

**Blood collection using the saphenous vein.**
Retrorbital plexus
The retrorbital plexus is a system of dilated venous channels at the back of the orbit. Blood can be collected from this area in anesthetized rats using a microhematocrit tube. There should be no movement of the head during the procedure. Press down with the thumb and forefinger just behind the eye and pull back on the skin to allow the eyeball to protrude. Position a microhematocrit tube along the inner corner of the eye (medial canthus) beside the eyeball. Insert the tube gently but firmly on the medial side through the conjunctiva towards the back of the eye along the orbit. Rotate the tube gently as you proceed. Blood should flow freely if the tube is properly inserted. Tilt the head slightly downward to improve flow. After collecting the blood withdraw the tube and apply pressure on the closed eyelids to stop any bleeding. Remove excess blood with gauze. Complications include damage to the eye and surrounding tissues.

Brachial vessels
Blood can be collected from the brachial plexus as a terminal procedure in deeply anesthetized rats. Make a cut through the skin at the side of the thorax into the angle of the forelimb (axilla) to expose the axillary vessels. Transect the vessels and allow blood to pool into the pocket created by tenting the skin. Aspirate the mixed venous arterial blood into an appropriate receptacle.

Vena cava and abdominal aorta
Blood can be obtained from the posterior vena cava or abdominal aorta in a deeply anesthetized rat following laparotomy. Approach the vessel at a shallow angle using a fine gauge needle attached to a small syringe. This is a terminal procedure.

Cardiac puncture
Up to 10 ml of blood can be obtained from the heart of a deeply anesthetized rat in a terminal procedure. The most common approach is to lay the rat on its back and insert a 22 to 20 gauge needle attached to a 3-10 ml syringe just behind the xiphoid cartilage and slightly left of the middle. The needle should be introduced at 10-30 degrees from the horizontal axis of the sternum in order to enter the heart. Alternatively approach the heart laterally immediately behind the elbow at the point of maximum heartbeat. The figures above show terminal blood collection by cardiac puncture in a deeply anesthetized rat.

Administration of substances
Materials to be administered to rats can be given orally (e.g. in water or feed) or injected systemically through a variety of routes. The average daily consumption of feed and water for an adult rat is 15-25 g and 30-50 ml respectively. The following volumes can be injected into rats safely: 2-5 mls subcutaneously, 0.1-0.2 ml intramuscularly (0.1 ml per site), 1.5-2.5 mls intravenously, 3-5 mls into the stomach and 3-5 mls intraperitonealy. Intramuscular injections are usually not recommended in rats because of the small muscle mass. A fine gauge needle should be used to make injections in the anterior thigh muscle.
Gavage
Oral gavage is performed using a ball ended feeding needle or a flexible feeding tube. Estimate the distance that the needle/tube needs to be inserted into the rat (usually from the nose to the first rib) and mark it on the needle/tube. Restrain with the rat extended in a straight line to facilitate introduction of the gavage needle. Introduce the needle in the space between the left incisor and molars. Gently direct it caudally toward the right ramus of the mandible. The rat usually swallows as the feeding tube approaches the pharynx facilitating entry into the esophagus. If the animal struggles or appears to be in respiratory distress withdraw the tube and begin again. Once the desired position is attained, inject the material and withdraw the syringe. Monitor the animal after the procedure to ensure that there are no adverse effects such as regurgitation or respiratory difficulty.

Subcutaneous injections:
Subcutaneous injections are usually made into the loose skin over the neck or flank using a fine gauge needle. Insert the needle 5-10 mm through the skin before making the injection. Lack of resistance to the injection is indicative that you are in the right location. Check for leak back especially if a larger volume is injected.

Intraperitoneal injections
Intraperitoneal injections are usually made in the lower right quadrant of the abdomen. The rat is restrained with its head tilted lower than the body to avoid injury to internal organs or major blood vessels. After swabbing the lower right quadrant with alcohol, a fine gauge needle is introduced slowly through the skin, subcutaneous tissue and abdominal wall. Withdraw the syringe plunger to ensure that you are not in the bladder or intestines. If nothing is withdrawn inject the material slowly. If you accidentally enter the bladder or intestines withdraw, discard the needle and syringe and start over.
Pictures demonstrating intraperitoneal injection in a rat

Intravenous injections
Intravenous injections are usually made into the dorsal tail vein. Warm the tail by immersing it in warm water or placing the tail under a heat lamp. The tail vein is easier to see in non-pigmented rats. A fine gauge needle should be used for this procedure.

Tail vein injection demonstrating warming the tail to dilate the vessels and making the injections

Hamsters
Hamsters are easily agitated and will bite quickly and deeply; consequently they should be approached gently and with caution until they become accustomed to being handled and familiar with the handler. Several methods may be useful in handling a hamster. Both hands may be cupped with one under the animal and the other on top. The hamster may be picked up with one hand in a manner similar to that of the rat: the index finger on one side of the neck and the middle finger on the other side so the palm of the hand is covering the dorsum of the hamster. The hamster can also be scruffed by grasping the loose skin over the neck and shoulder; however, this skin is very loose, and practice is necessary before this method can be used casually. It is sometimes easier for the occasional handler to use a cup when transferring hamsters from point to point. Techniques used in rats and mice can be modified for use in hamsters, except tail vein injections since hamsters have very short tails.

Gerbils
Gerbils respond to and are effectively handled by the general methods indicated for other small rodents. For rapid handling of a large number, lifting by the tail near the body is desirable. Avoid holding gerbils near the end of the tail since the skin near the tip is fragile and may slip off. Techniques used in rats and mice can be modified for use in gerbils.

Guinea Pigs
Guinea pigs seldom bite, but are timid or easily frightened and may squeal and make determined efforts to escape when being caught. They can also scratch if their nails are long. Once they are securely held they usually sit quietly. The key to handling guinea pigs is to handle them gently, make slow deliberate movements and avoid loud noises while you are handling them. Guinea pigs that are handled gently and often can become very tame.

Revised 1/6/09, updated 2/27/13, 10/20/15
To pick up a guinea pig move slowly until you are ready to grasp the guinea pig and then grasp quickly; they can move quickly despite their bulk. They are also quite heavy (the average pig weighs 600-700g) and may be hurt if they fall from any height. Despite their size, pressure around the thorax can cause damage and inhibit breathing, just like in smaller rodents.

If your hands are large enough, restrain the guinea pig with one hand (index finger and middle finger around the neck, the palm over the back and the other fingers grasping the body). Lift the front end of the guinea pig with this hand and then use the other hand to cup the hindquarters and support the weight. Supporting the weight is absolutely crucial when handling very large pigs or pregnant females. For those with small hands where holding the pig around the thorax with one hand might exert undue pressure, two hands can be used to restrain and transfer the pig, one hand on each side.

Once you have picked up the pig, restrain it comfortably against your body (facing you) with one hand under the hindquarters to support the weight. Special care should be exercised in handling pregnant females since they may become very heavy and awkward in late pregnancy.

Vocalization and Other Behaviors
Guinea pigs are very vocal. They quickly learn who brings food and will greet your arrival with a series of high-pitched squeaks. They will also "whistle" or "pur" appreciatively, and scream if they are frightened or upset. Other behaviors associated with fear, loud noises and sudden movements include 'freezing' and 'shivering'. They may also 'stampede' away from a perceived danger with little regard for their physical safety, damaging their legs or falling from heights if they are not restrained.

Rabbits
Rabbits don’t often bite, but can inflict painful scratch wounds, especially with their hind feet. Hold them in a way that directs their hind feet away from your body. Grasping the loose skin over the shoulder with the head directed away from the holder is the best method of initial restraint. When lifting, support the lower part of the body by the other hand. You should never lift rabbits by the ears. If the rabbit begins to struggle violently or develops rotational movement with the hindquarters, it should immediately be placed on a solid surface and calmed, even if that means releasing it on the floor. Continued violent struggling frequently leads to fracture of one or more lumbar vertebrae, and severe injury to the spinal cord resulting in the immediate need for euthanasia.

Rabbits can be placed in a state of hypnosis by gently rolling them on their back and slowly stroking the abdomen.

During restraint rabbits may exhibit sudden violent efforts to escape and in the process dislodge intravenous catheters, gavage tubes, etc., causing spills or otherwise endangering themselves or personnel. Consequently, it is essential that complete restraint be accomplished before attempting such procedures. Particularly important are mechanical restrainers such as the one shown and are strongly recommended for most procedures.
Injections and blood sampling in rabbits

**Intravenous.** Equipment: 20 to 25g needle of suitable length with syringe. A short bevel needle not more than 1 inch long and a syringe of 5 ml capacity or less is recommended. A rabbit holder (restrainer) of metal or plastic construction should be used. Do not attempt this procedure using manual restraint. Use a restraining device if working alone. Light anesthesia may also be used if working alone.

When using the rabbit ear, a low-wattage light bulb can be used for heat. This also aids by providing additional light. Frequently, brisk rubbing of the skin with a gauze sponge moistened with alcohol will produce adequate dilation of the vessel. An indwelling catheter can also be placed in the ear vein for repeated administration of substance or for collection of blood. The total blood collected at one time should not exceed 1% of body weight in kilograms for survival studies. Adequate time has to be allowed for the animal to recover the loss. See [Veterinary Recommendations for Multiple Blood Draws](#).

When collecting blood from the central auricular artery ensure that there is complete hemostasis. This may entail applying pressure to the artery for several minutes. It is also advisable to recheck the animal 15 min or so after the end of the procedure to ensure that there is no bleeding. A repeat check may also be necessary.

Cardiac puncture must be performed as a terminal procedure only. Anesthesia is required for this procedure. Use a 18-20 gauge 1.5 in needle and appropriate sized syringe. Either a lateral or sternal approach can be used.

**Intramuscular.** Equipment. 20 to 22g, 1 inch needles with a 1 to 5 ml syringe.

Injections can be made into the lower back muscles and cranial muscles of the thigh (preferred). An assistant is usually needed.

**Subcutaneous.** Equipment: 20 to 22g, ¾ to 1 inch needles with appropriate size syringe. Ensure adequate restraint to avoid injury to the rabbit.
## Agents and methods of euthanasia by species

<table>
<thead>
<tr>
<th>Species</th>
<th>Acceptable⁴</th>
<th>Conditionally acceptable⁵</th>
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</thead>
<tbody>
<tr>
<td><strong>Amphibians</strong></td>
<td>Barbiturates, inhalant anesthetics (in appropriate species), CO₂, CO, tricaine methane sulfonate (TMS, MS222), benzocaine hydrochloride, double pithing</td>
<td>Decapitation⁷ and pithing, stunning and decapitation</td>
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<tr>
<td><strong>Birds</strong></td>
<td>Barbiturates, inhalant anesthetics, CO₂, CO</td>
<td>N₂, Ar, cervical dislocation, decapitation</td>
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<tr>
<td><strong>Cats</strong></td>
<td>Barbiturates, inhalant anesthetics, CO₂, CO, potassium chloride in conjunction with general anesthesia</td>
<td>N₂, Ar</td>
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<tr>
<td><strong>Dogs</strong></td>
<td>Barbiturates, inhalant anesthetics, CO₂, CO, potassium chloride in conjunction with general anesthesia</td>
<td>N₂, Ar</td>
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<tr>
<td><strong>Fish</strong></td>
<td>Barbiturates, inhalant anesthetics, CO₂, tricaine methane sulfonate (TMS, MS 222), benzocaine hydrochloride, 2-phenoxyethanol</td>
<td>Decapitation and pithing, stunning and decapitation/pithing</td>
</tr>
<tr>
<td><strong>Nonhuman primates</strong></td>
<td>Barbiturates</td>
<td>Inhalant anesthetics, CO₂, CO, N₂, Ar</td>
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<tr>
<td><strong>Rabbits</strong></td>
<td>Barbiturates, inhalant anesthetics, CO₂, CO, potassium chloride in conjunction with general anesthesia</td>
<td>N₂, Ar, cervical dislocation (&lt; 1 kg), decapitation</td>
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<tr>
<td><strong>Reptiles</strong></td>
<td>Barbiturates, inhalant anesthetics (in appropriate species), CO₂ (in appropriate species)</td>
<td>Decapitation and pithing, stunning and decapitation</td>
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<tr>
<td><strong>Rodents and other small mammals</strong></td>
<td>Barbiturates, inhalant anesthetics, CO₂, CO, potassium chloride in conjunction with general anesthesia, microwave irradiation</td>
<td>Methoxyflurane, ether, N₂, Ar, cervical dislocation (rats &lt;200 g), decapitation</td>
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<td><strong>Ruminants</strong></td>
<td>Barbiturates, potassium chloride in conjunction with general anesthesia</td>
<td>Chloral hydrate (IV, after sedation)</td>
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<tr>
<td><strong>Swine</strong></td>
<td>Barbiturates, CO₂, potassium chloride in conjunction with general anesthesia</td>
<td>Inhalant anesthetics, CO, chloral hydrate (IV, after sedation)</td>
</tr>
</tbody>
</table>

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⁵ Acceptable methods consistently produce humane death when used as the sole means of euthanasia.
⁶ Must be scientifically justified and approved by the IACUC.
⁷ Individuals using guillotines for decapitation should ensure that the equipment is functioning properly.

Revised 1/6/09, updated 2/27/13, 10/20/15
Forms

Below are some of the important forms that are used regarding animal care and use. There are also links to various suggested forms.

Attached forms:

**Charge Authorization**
This form is required. It tells Animal Resources which account to pull money from to pay for services related to animal care and use.

**Census Sheet**
This is the blue form that will be hanging on the wall of the anteroom for each animal suite. Each investigator has their own census sheet. When animals arrive, they are added to the census sheet by the animal care staff. When a lab permanently removes an animal, the animal must be removed from the census by the lab or the lab will continue to be charged per diems. At the end of each month, the animal care staff will record the number of animals for each PI. If animals were removed and not taken off the census, per diems will be charged for that animal until the end of the month when the final count is recorded.

**Maintenance Log for Surgical Areas**
Each surgical area should have a log that indicates when the area was cleaned. A sample form is attached. The ACUC and/or USDA may request to see this form during an inspection.

**Room Environment Record**
This record can be found hanging outside each animal room. It is completed by the animal care staff and is available for investigators if there are any questions about room temperature, humidity, lighting, etc.

Links:

**Anesthesia record**
Animals must be monitored while under anesthesia. A sample anesthesia form is provided but each lab can design their own. The form must include the protocol number, PI, procedure done, anesthetic used, analgesic used date, time and when the animal recovered and was returned to the cage. Values regarding anesthetic level, monitoring equipment values (pulse ox, end tidal CO2, heart rate, etc.) should be recorded at least every 15 minutes. This form must be kept with the lab data. The ACUC and/or USDA may request to see these forms.

The suggested form is 2 pages long. It is easier to print or copy it double-sided.

**Post procedural monitoring**
Following a procedure/surgery, animals must be monitored. A sample monitoring form is provided but each lab can design their own. The form must include the protocol number, PI, procedure done, date, time and observations, suture removal if applicable and a pain assessment.
ANIMAL RESOURCES
G007 TRAYLOR
TEL NO 410-955-3713

CHARGE AUTHORIZATION

DATE:

Please provide me with an Animal Resources Job Number. I will be responsible each month for this job number’s tokens. Each month, Animal Resources will provide me with details of charges against this job number. Charges incurred by any user of this job number may be deducted from the following account, which I am authorizing to use.

University Account Number: _______________________________________________________

University Acc Expiration Date: ___________________________________________________

MO: DAY: YEAR:

Principal Investigator: ____________________________________________________________

Title: First: Last:

Address: _________________________________________________________________

___________________________________________________________________________

___________________________________________________________________________

Telephone number: __________________________________________________________

Signature: _________________________________________________________________

To continue using Animal Resources, fill out this form and return it to our office. The job number will be active 7 days after the form is received.

Please disregard this reminder if you have already sent in your new account number.
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<th>ID</th>
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IMPORTANT
PLEASE NOTE

When recording additions and subtractions, no more than one total figure per box should be recorded.

RESULT OF HEAD COUNT ON LAST DAY OF THE MONTH

______________________________
Counted by
# Maintenance Log for Surgical Areas

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<th>Date Cleaned</th>
<th>Date of Procedure</th>
<th>Surgery Table</th>
<th>Countertops</th>
<th>Surgical Lights</th>
<th>Floors</th>
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Comments:

Revised 1/6/09, updated 2/27/13, 10/20/15
# Room Environment Record

**Building:**  
**Room #:**  
**Month/Year:**  
**Species:**  

**Monthly room sanitation:**

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<th>Temp</th>
<th>Humidity</th>
<th>Light timer checked</th>
<th>Cage changed</th>
<th>Rack changed</th>
<th>Room cleaned</th>
<th>Health check</th>
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Suggested Readings


CDC (Centers for Disease Control) and NIH (National Institutes of Health). Biosafety in Microbiological and Biomedical Laboratories. 5th edition. 2007


Holzworth, J. Diseases of the Cat, medicine and surgery, Saunders Pub. 1987


**SURGERY, ANESTHESIOLOGY & ANALGESIA**


Edgerton, M.T. The Art of Surgical Technique, Williams and Wilkins, 1988


Fowler, M.E., Restraint and Handling of Wild and Domestic Animals, Iowa State University Press, Ames, Iowa, 1979


Piermattei, D.L., Flo, G.L. and Giddings, F.D. Handbook of small Animal Orthopedics and Fracture


The Department of Molecular and Comparative Pathobiology has some of these and many other publications available in their Departmental Library, located in Broadway Research Building 843. These items are not circulated but can be used in the library.