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Emergency Contacts

EMERGENCY VETERINARY CARE call the veterinary care pager –

1. 934-4656

OR

2. 231-6762

[Barry Robert](#), DVM
Director of Comparative Biology Core
Office: 763-0924
Pager: 934-4656
Long Distance Pager: 877-565-0301

[Cindy Kloster](#), RLATG
Assistant Director of Comparative Biology Core
Office: 763-2528
Pager: 231-6762
Long Distance Pager: 877-340-7119

FACILITIES MANAGEMENT

Electrical, Plumbing, Heating, Ventilation, Air Conditioning

[Bob McNeese](#)
Director of Facilities Management
Office: 763-2505
Emergency: 763-2508

SECURITY PBRC

Injury, Fire, Flood, Chemical Spill, Radiation
Office: 763-2508
Emergency: 763-2508
Pager: 930-9718

INSTITUTIONAL OFFICIAL

[Richard Rogers](#), Ph.D.
Associate Executive Director of Basic Research
Phone: 763-2577

Common Acronyms

AAALAC, Int.	Association for the Assessment and Accreditation of Laboratory Animal Care, International
ABSL II	Animal Biosafety level II
AV	Attending Veterinarian
AVMA	American Veterinary Medical Association
AWAR	Animal Welfare Act and Regulations
BSL II	Biosafety level II
CBC	Comparative Biology Core
HREA	Health Research Extension Act
HRM	Human Resource Management
IACUC	Institutional animal care and use committee
IBRDS	Institutional Biohazard and Recombinant DNA Safety Committee
IO	Institutional Official
NIOSH	National Institute of Occupational Safety and Health
PBRC	Pennington Biomedical Research Center
PHS	Public Health Service
PPE	Personal protective equipment
SOP	Standard Operating Procedure
<i>The Guide</i>	<i>Guide for the Care and Use of Laboratory Animals</i>
USDA APHIS	United States Department of Agriculture Animal Plant Health Inspection Service

Introduction

The Pennington Biomedical Research Center (PBRC) Comparative Biology Core (CBC) supports and promotes the humane, ethical, and responsible use of animals in research and teaching. Furthermore the CBC is responsible for and committed to the highest quality of animal care. Our policies and program of animal care and use are comprehensive and intended to promote full compliance with federal, state, and local regulations governing the care and use of laboratory animals. This manual describes our institutional policies and procedures for use of animals in teaching and research.

The PBRC is an assured institution of the Public Health Service (PHS) and therefore adheres to the PHS *Policy on Humane Care and Use of Laboratory Animals* and the United States Department of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS), *Animal Welfare Act and Regulations (AWAR)*. The Health Research Extension Act (HREA) of 1985, Public Law 99-158, “Animals in Research” (<http://grants.nih.gov/grants/olaw/references/hrea1985.htm>) provides statutory mandate for the PHS Policy. The PBRC has been accredited by Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC, Int.) since 1993 and this accreditation signifies verified compliance with the requirements for the proper care and treatment of all vertebrate laboratory animals, irrespective of species, location, investigator, use, or funding source as described in the *Guide for the Care and Use of Laboratory Animals*.

Investigators and all personnel involved in the use of laboratory animals should review the following documents – print copies are available in the CBC or online at the links indicated below.

1. The PHS *Policy on Humane Care and Use of Laboratory Animals* (<http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf>)
2. The USDA APHIS AWAR (<http://www.aphis.usda.gov/ac/publications.html>)
3. The *Guide for the Care and Use of Laboratory Animals* (<http://www.nap.edu/readingroom/books/labrats/index.html>).

Comparative Biology Core Personnel

Director

[Barry Robert](#), DVM, Ph.D., DACLAM

Office: E1002

Phone: 763-0924

Fax: 763-2579

Pager: 934-4656 Long Distance Pager: 877-565-0301

Assistant Director

[Cindy Kloster](#), BS, RVT, RLATG

Office: E1004

Phone: 763-2528

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Pager: 231-676 Long Distance Pager: 877-340-7119

Supervisor

Linda Chase, RLAT

Phone: 763-2624

Clerk

Vacant

Office: E1003

Phone: 763-2573

Fax: 763-2579

Laboratory Animal Technicians

Phone: 763-2624

CBC personnel are available on weekdays between 7:30 a.m. and 4:30 p.m.

Physical Facilities

The CBC operates and maintains approximately 38,000 ft² of animal housing and support space. This space includes:

- ☆ CBC offices – E1002, E1003, E1004
- ☆ Conventional animal housing
- ☆ Barrier animal housing
- ☆ Laboratory animal support space and storage
- ☆ Transgenic core laboratories
- ☆ Behavioral study space
- ☆ Surgical suites and surgical support
- ☆ Procedural laboratories
- ☆ Necropsy facilities

Services

The Comparative Biology Core is a service-oriented unit of the PBRC. Our primary role is to provide animal care and research support services to investigators at the Center and maintain compliance with applicable regulations governing the care and use of animals in research.

Animal care

- ☆ Daily animal husbandry and care
- ☆ Environmental enrichment
- ☆ Dietary support
- ☆ Animal transport

Administration

- ☆ Animal ordering, receipt, and disbursement
- ☆ Oversight of physical facility maintenance and environmental control
- ☆ Space utilization/allocation
- ☆ Animal shipment

Training

- ☆ General animal use orientation
- ☆ Animal handling
- ☆ Specific animal research technical training
- ☆ Surgery training

Veterinary Medical Care

- ☆ Laboratory animal medical care
- ☆ Surgical services and training
 - Sterilization of surgical instruments
 - Aseptic technique
 - Anesthesia

- Surgical technique
- Postoperative recovery and care
- Analgesia
- ☆ Animal use protocol development
- ☆ Assistance with animal model development
- ☆ Clinical pathology and sample collection
- ☆ Biopsy and necropsy

Other Technical Assistance

- ☆ Specific technical assistance is available to investigators from the CBC laboratory animal care technicians with prior approval by the assistant director.
- ☆ Requests for technical assistance for services outside the normal husbandry routine or for services related to one's research project must be made in writing on a Procedure Request Form ([Appendix A](#)) and returned to the assistant director for approval.
- ☆ Comparative Biology may assess a minimal charge to recover labor costs.

General CBC Guidelines

- ☆ Eating and drinking are prohibited in the CBC except in the administrative offices and E1005 (staff break room).
- ☆ Only closed toed shoes with non-skid soles should be worn into CBC.
- ☆ All persons entering Comparative Biology Core animal rooms must wear appropriate personal protective equipment.
 - Laboratory coats are available in the CBC foyer as personnel enter.
 - Required PPE in animal rooms includes a laboratory coat, gloves, hair bonnet, and surgical or NIOSH mask.
 - Gloves, surgical bonnets, and masks are available in each animal room.
- ☆ Do not bring personal belongings such as purses or backpacks into animal rooms.
- ☆ Portable radios are not allowed in the animal facility. However, personal devices such as IPODS and Walkmans can be worn in the facility with headphones set at a low volume. If individuals standing near you can hear the sound from the headphones it is too loud and will disturb the animals.
- ☆ If you bring a cart into CBC you must spray its wheels with quaternary ammonium (available in spray bottle in the foyer) before entering.
- ☆ The director or assistant director must approve equipment items other than carts before it is brought into CBC.

Institutional Animal Care and Use Committee

Regulatory Authority

The PBRC Institutional Animal Care and Use Committee (IACUC) is responsible for the oversight of all animal care and use at the Center as is mandated by the PHS *Policy on Humane Care and Use of Laboratory Animals* and the USDA *AWAR*. IACUC oversight is largely achieved through the protocol review process, review of the Center's programs for humane care and use of animals, and inspection of the CBC.

Committee Function

Specific committee duties include:

- ☆ Review of proposed use of animal in research and/or teaching (protocol review)
- ☆ Inspection of the animal facilities (all sights where animals are housed and/or used in experiments)
- ☆ Evaluation of institutional programs related to animal use (i.e. Occupational Health and Safety, Biosafety, personnel training, etc.)
- ☆ Submission of reports to the responsible institutional official
- ☆ Establishment of a mechanism for receipt and review of concerns involving the care and use of animals at the Center.

In performing these duties the IACUC assures consistency with the PHS *Policy on Humane Care and Use of Laboratory Animals*, the USDA *AWAR* and the *Guide*. The Institute for Laboratory Animal Resources, National Research Council publishes the *Guide* with the stated purpose:

“to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate. The *Guide* is also intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles.”

IACUC Meeting Schedule

IACUC meetings are generally held on the third Thursday of each month ([Current Schedule](#)) and protocols are due in to the IACUC administrator in B1022 by noon on the preceding Friday. The committee will communicate the outcome of the review of their protocols or amendments in writing to all investigators.

Committee Composition

The Executive Director appoints IACUC members based on the requirements of the PHS *Policy on Humane Care and Use of Laboratory Animals*, the USDA *AWAR*. The IACUC is composed of research scientists who are familiar with the use of animals in research, the institutional veterinarian, the PBRC safety officer, and a community member who is in no way affiliated with the institution to represent the general community interest with the use of animals in research. Investigators are encouraged to seek the advice of IACUC committee members during animal use protocol preparation and other aspects of their animal experiments. An organization chart and current IACUC roster are included in [Appendix B](#) and [Appendix C](#) respectively.

Protocol Review

All research and/or teaching activities requiring the use of laboratory animals must be reviewed and approved by the IACUC **prior** to commencement to ensure regulatory compliance. This animal use

may be associated with a grant or contract application, collaboration with investigator at this or another institution, or training procedure. IACUC approval is achieved via submission of an investigator-initiated Protocol for Animal Care and Use to the IACUC for review. The [Protocol for Animal Care and Use form](#) is available on PINE. Instructions for completion and submission of the form are included with the form. Investigators are encouraged to seek the advice of IACUC members and/or the attending veterinarian (AV) questions concerning completion of the protocol form.

The *Guide* recommends that the following topics be considered (and addressed) in the preparation and review of animal care and use protocols:

- ☆ Rationale and purpose of the proposed use of animals.
- ☆ Justification of the species and number of animals requested.
- ☆ Availability or appropriateness of the use of less-invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A of the *Guide*, "Alternatives").
- ☆ Adequacy of training and experience of personnel in the procedures used.
- ☆ Unusual housing and husbandry requirements.
- ☆ Appropriate sedation, analgesia, and anesthesia. (Scales of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A of the *Guide*, "Anesthesia, Pain and Surgery.")
- ☆ Unnecessary duplication of experiments.
- ☆ Conduct of multiple major operative procedures.
- ☆ Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
- ☆ Post procedure care.
- ☆ Method of euthanasia or disposition of animal.
- ☆ Safety of working environment for personnel.

Protocol Amendments

During the three-year "life" of an approved protocol investigators may need to adjust, amend, or modify portions of the protocol. Minor changes to an active protocol can be made by a protocol amendment. Examples of items appropriate for the amendment process include:

- ☆ Change in drug dosages
- ☆ Minor changes in the number of animals needed
- ☆ Changes in the number or designation of experimental groups
- ☆ Changes in duration or time points of the experiment

Protocol amendments can generally be accomplished by sending a memo to the IACUC requesting the change. The protocol amendment must address the specific change or deviation from the original protocol, the rationale for the change or deviation, and whether or not additional animals will be necessary. See [Appendix D](#) for an example amendment that can be used as a guide.

Significant changes to a protocol require a complete rewrite of the protocol. Examples of these include:

- ☆ Changes in the objective of the study
- ☆ Switch from a nonsurvival to survival surgery
- ☆ An increase in the degree of invasiveness of a procedure or discomfort to an animal, addition of a painful procedure
- ☆ Change in species of animal used
- ☆ Significant increase in the number of animals needed

For questions concerning the appropriateness of an amendment or the need to write a completely new protocol please consult with the IACUC Chair or the attending veterinarian.

Protocol Continuing Review

Each July the IACUC administrator sends out protocol continuation/renewal forms to all investigators with active protocols. The investigator must complete the form and return it to the IACUC for review. The form is then reviewed by the IACUC.

Three-year de novo Review

As mandated by the PHS *Policy* and *AWAR* a Protocol for Animal Care and Use can only be approved for three years. At the end of three-year period an updated protocol must be submitted for full review by the IACUC. The IACUC administrator will contact the investigator 60 days prior to protocol expiration notifying him/her of the upcoming expiration. The updated protocol should include changes in study objectives, justification of animal numbers, etc.

Program and Facility Review

Semiannually the IACUC performs a thorough review of all aspects of animal care and use at the Center. This review includes review of all programs related to animal use and a physical inspection of all areas of animal housing and use. After the inspection persons responsible for correction of the deficiencies are notified of the deficiency and a deadline for correction of the deficiency. A report of their findings and a schedule to correct any deficiencies is also submitted to the institutional official.

Occupational Health and Safety Program

Participation in the Pennington OH&S Program is required for all personnel working in the CBC. The three components of the OH&S program are; completion of a health questionnaire upon employment, current tetanus immunization, and CBC orientation. Upon employment all individuals that will have contact with laboratory animals must complete a short confidential health questionnaire. Our occupational health physician at the LSU Student and Employee Health Service on the LSU Campus reviews the questionnaire. Based on this review the physician may elect to set up a visit with the employee to further evaluate and advise him/her of individual risk factors while working with laboratory animals. Furthermore, all individuals working with laboratory animals are required to have a current tetanus immunization. During CBC laboratory animal orientation personnel are advised of the general risks of working with laboratory animals in the CBC including, laboratory animal allergies

([Appendix E](#)), injuries, bite wounds, zoonotic diseases, and physical hazards.

Reporting Animal Care Concerns

Comparative Biology is committed to the humane care and use of laboratory animals as set forth by the PHS *Policy*, USDA *AWAR*, and the *Guide*. Inappropriate or inhumane treatment or handling of laboratory animals will not be tolerated. All personnel are encouraged to report any concerns of inappropriate or inhumane care and use of laboratory animals to the IO, IACUC chairman, and/or the director of CBC. The confidentiality of the person reporting a concern or complaint will remain anonymous, is protected, and does so without penalty or fear of reprisal. All such reports will be investigated thoroughly. The complainant will be notified of the outcome of the investigation and any corrective action taken when necessary.

Security

Security in the CBC is very important for the health and safety of personnel and our research animals. Therefore access to the CBC is restricted by card-key access under computer control by the PBRC security. Access to animal rooms is restricted by key entry to those individuals whose animals are housed there. Animal room keys can be checked out through the Assistant Director's office (E1004). Personnel with access must not lend their card-key out allowing others access to the CBC.

To obtain card-key access to CBC, personnel that use animals in their research programs must:

1. Participate in the institutional Occupational Health and Safety Program by completing an occupational health questionnaire and submitting it to the Safety Office/HRM Department.
2. Investigators and research technicians are required to participate in a vaccine program.
3. Complete PBRC Animal Care and Use Orientation Program. The orientation program is held every other Thursday at 1PM in the CBC Rm E1005. Call Cindy Kloster at 3-2528 for scheduling.

All **visitors** to CBC must have prior approval from the director or assistant director of Comparative Biology or the IO.

Photography within the Comparative Biology Core is forbidden without prior approval of the Director or Assistant Director.

Lost keys and IDs should be reported immediately to the assistant director and PBRC Security.

Veterinary Medical Care

Consistent with the *Guide*, the responsibility for, and provision and oversight of veterinary medical care to the research animals in the CBC are the responsibility of the attending veterinarian or his designee. The CBC veterinary staff implements a program of veterinary care that encompasses preventive medicine including the rodent health surveillance program; clinical care including the management of spontaneous as well as protocol-associated health concerns; surgical and post surgical

oversight including anesthesia, surgery, analgesia, postoperative care; and euthanasia.

Preventive Health Program

Rodent Quarantine and Conditioning

- ☆ Upon arrival all animals undergo a health check by the receiving animal care technician and are placed in quarantine.
- ☆ The quarantine period is designed to assure the safety and well being of the existing colonies in the CBC and to allow an acclimation period for arriving animals.
- ☆ Animals from routine commercial vendors generally have a 7-day quarantine period.
- ☆ In the event there is a problem with shipment such as long delays in airports, damaged boxes, or dead animals in the box, the remaining animals will be held for a full quarantine period of 8 – 12 weeks until health status has been determined or in some cases the animals may be euthanized.
- ☆ Animals from non-routine vendors and collaborator institutions are generally quarantined for 8 – 12 weeks while their health status is verified.

CBC Health Surveillance Program

- ☆ The CBC health surveillance program is designed to address and maintain colony health as opposed to individual animal health.
- ☆ Many infectious diseases of rodents produce subclinical infections that do not produce overt disease in infected animals but may have an impact on research ([Appendix F](#): Rodent Health Chart).
- ☆ Sentinel animals are maintained in each animal room to detect the presence of infectious agents in our rodent colonies. Quarterly, serum from the sentinel animals is tested for serologic evidence of pathogens.

Vendor Health Surveillance Program

- ☆ Commercial vendors supply health surveillance information on their animal colonies with each shipment of animals.
- ☆ The CBC staff reviews this health information regularly.
- ☆ Investigators receiving animals from non-commercial sources (i.e. collaborators at other institutions) can expect the CBC staff to request current health surveillance data from the institution.

Policy on Rodent Contact Outside of CBC

To prevent the spread of diseases from rodents outside CBC, the CBC strongly advises the following policies.

- Do not keep rodents as pets if they work with animals in the CBC.
- Do not handle rodents in other animal facilities.
- Do not handle rodents at pet shops.
- Do not handle feeder rodents for snakes.
- If you are exposed to rodents (i.e. in another animal facility, feral rodents in a barn or agricultural setting) do not enter CBC for at least 48 hours post exposure.

Clinical Medical Care

Animal Treatments

- ☆ The veterinary staff makes regular rounds of the animal facility and examines sick animals reported by the CBC staff.
- ☆ The principal investigator or his/her designee is generally contacted prior to the initiation of treatment to discuss the diagnosis, prognosis, treatment, and/or alternative actions.
- ☆ Veterinary care standards require that sick animals be provided veterinary care. If the investigator would prefer not to treat the animal it must be promptly utilized in a terminal experiment or be euthanized.
- ☆ If the principal investigator or a representative cannot be reached within a reasonable period of time, the veterinary staff will initiate treatment until contact is made.
- ☆ Investigators that recognize animals with health concerns should also report those to the veterinary staff by completing a Laboratory Animal Medicine Clinical Sheet ([Appendix G](#)).

Diagnostic Services

- ☆ The CBC submits diagnostic samples to the Division of Laboratory Animal Medicine at the LSU, School of Veterinary Medicine, and the Research Animal Diagnostic and Investigative Laboratory at the University of Missouri.
- ☆ Services available at these laboratories include microbiology, parasitology, pathology, clinical pathology and serology.
- ☆ Investigators interested in any of these services should contact the Director or Assistant Director for contact information.

Surgery

The attending veterinarian is responsible for oversight of all surgical programs at the Center. All survival surgeries must be performed in the CBC surgical facilities. The CBC Surgery Guidelines ([Technical Resources](#)) describe the expectations for pre-surgical, surgical, and post surgical care. The veterinary staff is available to assist and/or train investigative staff in all aspects of surgery including aseptic technique, anesthesia use, anesthesia monitoring, surgical techniques, analgesia, and postoperative care. A surgery orientation is available for all investigators and is mandatory starting in November '05 for all new personnel that have surgical procedures in their animal protocols. Contact the assistant director or director to schedule surgical assistance or training.

Careful planning of all phases of surgical procedures by the research team will optimize successful outcomes.

Surgical Support in CBC

1. The CBC maintains a 3 suite surgical facility – E1059, E1060, and E1061.
2. Each surgery room is equipped with isoflurane anesthetic capabilities (induction boxes and anesthetic masks), warming pad surgical surfaces, glass bead sterilizers, and warmed recovery chambers.
3. Additional surgical supplies available in the CBC include:
 - a. Aseptic prep materials – gloves, clippers, Nolvasan scrub and alcohol basins with gauze.
 - b. Surgeon's items – sterile gowns and gloves, masks, and caps.
 - c. Sterile instrument packs, drapes etc. Surgical packs are only for use in surgery in rooms

- E1059, E1060, and E1061. Items borrowed should be returned clean and free of blood.
- d. Drugs – Anesthetics, analgesics and antibiotics are available from the assistant director of CBC.
- ☆ Anesthetics available: isoflurane, ketamine/xylazine/acepromazine cocktail^{CS}, ketamine/medetomidine^{CS}, and Nembutal^{CS}.
 - ☆ Analgesics available: bupivacaine/lidocaine combination, buprenorphine^{CS}, carprofen, ibuprofen, and acetaminophen.
 - ☆ **CHLOROFORM and ETHER use is FORBIDDEN** in the CBC.
 - ☆ **CS = controlled substances, see information below.**

Surgical Planning Considerations

- ☆ **Location** – Schedule CBC surgical suites via the signup sheet outside Rm 1027.
- ☆ **Presurgery preparation** – Are the surgical procedures described in your approved Protocol for Animal Care and Use? Do you have the necessary anesthetic and analgesic drugs (see Technical Resource section)? Do you have the proper surgical instruments? Do you have any specialized equipment needs? Do you have the appropriate suture materials?
- ☆ **Surgical procedure** - Are your instruments sterilized? Do you have sterile gloves? Provisions to do surgical prep of animal? Documentation of anesthesia and surgical procedure?
- ☆ **Postoperative care** – Are recovery procedures optimized and documented? Do you have the appropriate and necessary analgesic drugs?
- ☆ **Surgical records** - Documentation of surgical procedures is the responsibility of the investigative team. The research staff should maintain records of anesthesia, the surgical procedure, analgesics, and postoperative care. An example of a simple Rodent Surgical Record Sheet is available in [Appendix H](#).

Isoflurane Warning: Unnecessary exposure of personnel to isoflurane should be avoided. The Comparative Biology Core gas anesthesia machines use isoflurane gas that can pose a health risk to pregnant women. As recommended by OSHA, women in their first trimester of pregnancy should not be exposed to this anesthetic agent.

Controlled Substances

The Comparative Biology Core provides controlled substances for rodent anesthesia and/or euthanasia. Principle investigators must have IACUC approval to obtain controlled substances from CBC. The use of controlled substances is regulated by the Drug Enforcement Agency and therefore investigators using these agents must adhere to strict guidelines and be properly trained. The CBC has information (see Anesthetic/Analgesic Factsheets in Surgery Guidelines) on the anesthetics, analgesics, and euthanating agents available for dispensing. If you have not been properly trained in the use of the chemical agent or technique required in your protocol, contact Comparative Biology and request assistance. Do not attempt to use these agents unless you are sure you know how to safely administer them.

Guidelines for use of controlled substances:

1. **Sign out procedure:** The principle investigator or his designee must sign out controlled drugs from CBC along with appropriate log sheet.

2. **Log use:** As drug is used, the drug log must be completed accurately and signed by the user ([Appendix J](#)): Controlled Substance Log Sheet for example of a correctly completed sheet).
3. **Storage:** Controlled substances must be kept in a locked cabinet when not being used.
4. **Return:** When the bottle is empty (or if its use is discontinued) the bottle along with the completed log sheet must be returned to Comparative Biology.
5. **Monitoring:** The records and storage areas will be checked periodically to insure that the proper guidelines are being followed.

Failure to follow the controlled substance guidelines will result in the loss of privileges to obtain controlled substances from CBC.

Euthanasia

Euthanasia literally means “good death.” In the research setting it refers to the act of causing the death of a research animal in a manner that induces minimal pain and distress. The *PHS Policy, AWAR*, and *Guide* require that we follow the recommendations of the 2000 Report of the American Veterinary Medical Association Panel on Euthanasia ([Appendix I](#)). The method(s) of euthanasia used must be included in an investigator’s IACUC protocol. Methods that do not conform to the recommendations of the AVMA Panel on Euthanasia Report must be scientifically justified and approved by the IACUC.

A secondary method of euthanasia is also required to assure that death has indeed occurred. Appropriate secondary methods are the creation of a pneumothorax, cervical dislocation, or decapitation after the animal has undergone the primary method of euthanasia.

Animal Carcass Disposal by CBC Personnel

- ☆ Animals that die and are found by the animal technicians will be removed and placed in a biohazard bags that will be labeled with the name of the investigator, identity the animal, and the date and time the animal was found.
- ☆ Unless instructed otherwise, the carcass will be stored in the refrigerator in room E1027 for 72 hours, before disposal. If investigators would like the animals to be kept longer than 72 hours, special arrangements must be made by contacting the CBC Supervisor or Assistant Director.

Animal Carcass Disposal by Research Personnel

- ☆ Animals that are euthanized at the end of a study must be placed in a plastic bag of suitable size and strength.
- ☆ Animals infected with pathogenic organisms should be placed in biohazard plastic bags and labeled.
- ☆ Animal carcasses for disposal should be stored in the freezers in E1040A, E1069, E1118 or C1002.
- ☆ Animals that have been contaminated with radioisotopes **cannot** be disposed of in the CBC and instead must be handled as specified in the investigator’s Protocol for Animal Care and Use and approved through the LSU Radiation Safety Office.
- ☆ Animal carcasses that are being held for necropsy may be stored in the small refrigerator in E1027

Animal Orders

All animals entering the Pennington Biomedical Research Center must be ordered through the CBC. An approved IACUC Protocol for Animal Care and Use is required **prior** to ordering animals.

Commercial Vendor Orders

1. Fill out the Animal Order Form ([Appendix K](#)) completely and submit it to the CBC Assistant Director between 7:30 a.m. and 4:00 p.m., Monday through Friday.
 2. To ensure prompt delivery and adequate housing, requests for animals should be made a minimum of one week, prior to delivery.
 3. Rodent orders are placed before noon on Tuesday of each week.
 - The date of delivery is contingent on space availability in CBC and animal availability from the vendor.
 - Once orders are placed investigators are notified of the anticipated arrival date and housing location for the animals once out of quarantine.
 - There are no weekend or holiday deliveries.
- ☆ **Standing orders** will be set up by Comparative Biology (with the assistance from your Business Manager) whenever possible to minimize paperwork and ensure availability of animals of specified strain, age, and sex from a particular vendor.
- ☆ Standard commercial vendors include: Charles River Laboratories, Harlan, Jackson Laboratories, Taconic, and Myrtle's Rabbitry.

Non-commercial Source Orders

- ☆ All investigator-initiated requests for animals from scientific collaborators must be coordinated and approved through the Assistant Director of CBC.
- ☆ The Assistant Director will coordinate shipment of the animals from the supplying institution.
- ☆ Investigators receiving animals from non-commercial sources (i.e. collaborators at other institutions) can expect the CBC staff to request current health surveillance data from the institution prior to receiving the animals.
- ☆ The quarantine period for animals from non-commercial vendors is generally 8 – 12 weeks depending on the health status and health information provided by the supplying institution.

CBC Animal Ordering Tips

- Prior to placing an order CBC recommends that animal users discuss their animal procurement with the Assistant Director of CBC, this is especially important for first-time orders. The Assistant Director can offer advice on rodent vendors, the availability and costs for selected species/strains, and help avoid delays due to insufficient information on the request.
- Only official Animal Order Forms will be accepted; e-mail and verbal orders cannot be taken! An appropriate account number must be included on the form before animals will be ordered and this account will be used for per diem charges.
- Comparative Biology can assist investigators in selection of vendors for procurement of research animals.
- Although cost of the animals will certainly be considered it should not be the most important factor in vendor selection.
- Vendor selection criteria:

- The quality and suitability of the animal for the research program in which the animal will be used.
- The ability of the supplier to provide a steady supply of the required genotype/phenotype of animals necessary to complete the research project
- Colony health surveillance information available from the supplier.
- Transportation capabilities/arrangements of the vendor.
- Previous experience with vendor.
- Evaluation of vendor's facilities and programs for supplying animals

Animal Receiving

All incoming animals are received and inspected by CBC technicians. All incoming animals are housed in quarantine (see [Rodent quarantine and conditioning](#)) upon arrival. If abnormalities are detected in the animals during this period the investigator will be notified and Comparative Biology will contact the vendor in an attempt to obtain reimbursement. This is particularly true when laboratory testing is available indicating that the animals were diseased upon arrival. Animals harboring, or suspected of harboring a contagious disease will be isolated from the rest of the group and the principle investigator will be notified immediately. Any treatment deemed necessary will be discussed with the Principle Investigator prior to administration.

Animal Shipping

Investigators often request that animals be shipped to their collaborators at other institutions. All animal shipments must be coordinated through the CBC. Please notify the Assistant Director well in advance of pending shipments (minimum 2 weeks) to ensure that all necessary paperwork, shipping containers and shipping arrangements are complete. A Material Transfer Agreement (MTA) must be in place before shipping animals. Contact [Anne Jarrett](#) (3-2515) concerning MTAs. Animal shipments are regulated by State and Federal laws, and require veterinary examination and approval prior to shipment. All costs incurred for shipping animals (animal shipping boxes, gel packs, USDA Health Certificates, shipping fees, and international charges) are the responsibility of the investigator or his/her collaborator.

Animal Housing and Maintenance

The PBRC is a PHS-assured institution and the CBC is AAALAC accredited requiring us to maintain uniformly accepted standards of animal care described in the *Guide for the Care and Use of Laboratory Animals*. All research animals at the Center must be housed in the CBC and will be cared for by CBC personnel.

Animal Room Space

- ☆ The Assistant Director is responsible for determining where animals will be housed.
- ☆ Animals are generally housed in rooms according to species, source, and when space allows by investigator.
- ☆ The decision regarding the location of groups of animals is based on the environmental needs of the animals and any special requirements essential to the experimental plan.
- ☆ Animal room space is **not** permanently assigned.

- ☆ **Animals must not be moved from one room to another or between investigators without submitting an Animal Transfer Form ([Appendix M](#)) and obtaining approval from Comparative Biology.**

Animal Room Maintenance

- ☆ Clean, orderly, simply furnished animal rooms are essential to facilitate sanitization and vermin control.
- ☆ Investigators may not store supplies, equipment, and other material in animal rooms without the prior approval of the Assistant Director.
- ☆ Surgery and/or necropsies must not be performed within animal rooms.
- ☆ Minor procedures such as injections or collection of samples (i.e. blood collection) may be conducted in animal rooms or in nearby procedure rooms.

Procedure Room Use

- ☆ Comparative Biology maintains a number of procedure rooms for use by investigators.
- ☆ Investigators are encouraged to use the sign up sheets maintained outside the respective rooms to ensure availability.
- ☆ Rooms E1040A and B may be used for minor procedures (i.e. blood collection), euthanasia, tissue harvest, and necropsies.
- ☆ Room E1055 housed the NMR for use with mice and also may be used for minor procedures.
- ☆ Room E1069 is a necropsy room and may also be used for euthanasia and tissue harvest.
- ☆ Room E1018 may be used for minor procedures and behavioral testing.
- ☆ Room E1118 (in barrier) may be used for minor procedures and euthanasia.
- ☆ Room C1002 may be used for minor procedures, euthanasia, tissue harvest, and necropsy.

Animal Caging

- ☆ Space requirements of various species are outlined in the *Guide* ([Appendix L](#)).
- ☆ Housing that inconsistent with space recommendations of the *Guide* must be scientifically justified and approved by the IACUC.
- ☆ All animal cages and equipment are part of a central institutional pool, which is administered, maintained, and serviced by Comparative Biology.
- ☆ In cases where investigators must purchase specialized caging for their experiments the Director or Assistant Director of Comparative Biology must approve the cages prior to purchase.
- ☆ Uniformity, versatility, efficiency, interchangeability, and sanitation are all considered when procuring equipment that will be used in the CBC.

Animal Identification

- ☆ Each cage of animals must be identified by use of a cage identification card at all times.
- ☆ Minimal information recommended by the *Guide* includes the principle investigator's name, protocol number, species, strain, sex, date of birth, arrival/weaning/received date, and telephone number.
- ☆ Comparative Biology maintains a supply of cage cards for investigator use.
- ☆ Mice and rats may be individually identified by the use of ear punches, ear tags, or microchips.
- ☆ Rabbits are identified individually by tattoo or ear tags.
- ☆ Rabbit cage cards and health records must be kept for a minimum of 3 years post study for USDA inspections.

Animal Diets and Bedding

- ☆ The Comparative Biology Core stocks three Purina Rodent Chow formulations ([LabDiet® - PMI Nutrition International Home Page](#))
 1. 5001, Standard Rodent Chow
 2. 5015, Mouse Breeder Chow
 3. 5012, Rat Breeder Chow
- ☆ Special diet formulations can be purchased through approved vendors or mixed in the diet preparation room in “F” building.
- ☆ Diets that are specially formulated must meet dietary guidelines prescribed by the “*Guide*” to be palatable and nutritionally sound to maintain species dietary requirements.
- ☆ Food that does not meet the *Guide* criteria must be scientifically justified and approved through the IACUC.
- ☆ **Corncob bedding** is the standard contact bedding used for all rodent cages.
- ☆ **Diet prep room F1002B** is equipped for preparing special diets.
- ☆ General rules for use of **diet prep**:
 - Schedule in advance by contacting Cindy Kloster at ext. 3-2528.
 - Personnel using diet prep are responsible for cleaning up after use.

Environmental Enrichment

- ☆ The *Guide* recommends that environmental enrichment be provided to all research animals.
- ☆ The CBC provides three forms of enrichment for rodents:
 - Rodents are considered social animals and the *Guide* recommends group housing social animals whenever possible. Deviations from social housing must be scientifically justified and approved by the IACUC.
 - Animals are provided with materials to “nest” in. This may be in the form of Tek-Fresh bedding or nestlets added to the standard corncob cage bedding.
 - PVC conical tubes are provided to animals as hiding places.

Environmental Controls

- ☆ Temperature
 - Individual animal rooms are generally maintained at 22 – 24 °C.
 - Temperatures outside of this range should be approved by the IACUC.
 - Request for temperature changes to an animal room made 48 h in advance through the Comparative Biology office with a Procedure Request Form ([Appendix A](#)).
- ☆ Lighting
 - Each animal room is on an independent light timer so that the photoperiod can be set as dictated by the study.
 - Generally, lights are set at a 12/12 light cycle to ensure proper health of the animals.
 - Deviations from the 12/12 intervals must be approved through the IACUC.
 - Request for adjustment to light cycles must be submitted, in writing, to the Comparative Biology office 48 h in advance with a Procedure Request Form ([Appendix A](#)).

Vermin Control

- ☆ Control of roaches and other vermin is a serious problem for the operation of animal research facilities.
- ☆ Continual surveillance and rigid sanitation are necessary to minimize the encroachment of pests.

- ☆ To aid in vermin control efforts, the storage/maintenance of equipment and supplies in the animal rooms must be kept to a minimum.
- ☆ Due to the potential effects of insecticides on experimental animals Comparative Biology has a policy of NOT using insecticides near or in animal rooms unless it is absolutely necessary and then, only after consultation with the investigator.

Animal Transport

- ☆ When transporting animals **within the CBC** cages must be covered with a microisolator lid prior to movement out of the animal room.
- ☆ Transporting animals **outside of the CBC**:
 - IACUC approval is necessary to transport animals out of CBC to investigator laboratories.
 - When transporting animals to the laboratory cages must be covered with microisolator lids, placed on a cart and the cart shrouded with an opaque covering.
 - Drape material is available on a cart in the CBC vestibule.
 - During transport avoid heavily traveled public routes and use freight elevators to minimize the public's exposure to allergens.
 - Empty caging must be covered/shrouded during transport back to CBC.
 - Animal carcasses should be returned to one of the necropsy freezers in CBC for disposal.
 - Radioactive carcasses must be held in approved freezers for radioisotope decay.

Animals must not be housed in the laboratory overnight.

Per Diems and Animal Accounting

- ☆ Per Diem rates are set by the institution and based on projections of the number of animals housed in the animal facility on a daily basis over a fiscal year.
- ☆ A daily animal census is necessary for each animal room so that investigators are only charged for those animals present.
- ☆ Comparative Biology personnel do a complete count of animals in each animal room **every two weeks**.
- ☆ During the intervening period animals that are weaned, die, or are euthanized should be recorded on the room white board by the research staff to ensure per diem charges are current and accurate.
- ☆ Comparative Biology staff use the numbers recorded on the room white board to make the changes on the daily census log.
- ☆ Examples:
 - **Weaning animals:** If you wean 20 mice that are housed 4/box it should be recorded on the white board as,

+20 mice MH, + 5 boxes - P69
 - **Culling animals:** If you cull 5 single housed rats it should be recorded on the white board as,

-5 rats SH - 5 boxes - P29.
- ☆ Alternate per diem or room charges will be determined as the need arises for experimental protocols that require exclusive use of a room or area, or studies requiring specialized housing.

Transfer of Animals Between IACUC-Approved Protocols

All transfer of animals between protocols whether between two protocols held by the same investigator or held by different investigators must be properly documented.

1. Complete an Animal Transfer Form ([Appendix M](#)).
2. Submit the form to the Assistant Director of CBC for approval.
3. The Assistant Director must approve all animal transfers and changes in housing location associated with transfers.

Use of Hazardous Materials in Comparative Biology

Biohazards

- ☆ The CBC has the facilities to manage biosafety level II agents.
- ☆ Investigators who plan to use biohazardous agents in animals must obtain prior approval from the Institutional Biohazard and Recombinant DNA Safety Committee (IBRDS) and IACUC.
- ☆ The IBRDS and IACUC protocols must describe all required safety and containment procedures.
- ☆ The PBRC safety officer and the Director and Assistant Director of Comparative Biology can offer assistance in completion of these protocols.
- ☆ General guidelines for BSL II agents use:
 1. All researchers and their staff must submit an Occupational Health form including the proposed agent used in their experiment to the HRM department. This form is submitted to the occupational health physician for authorization to participate with the use of this agent.
 2. The physician will make recommendations pertaining to health of the individuals proposed to work in the BSL II study.
 3. All individuals must be fit tested for NIOSH 95 respirators before participating in the approved protocol.
 4. All areas that contain BSL II agents must have appropriate room signage completed ([Appendix N](#)) and placed on the door to the animal room.
 5. All animals that have been inoculated with infectious agents must be so labeled and the carcasses disposed in accordance with the IBRDS-approved protocol.
 6. The information necessary to complete these studies will be compiled in a Standard Operating Procedure and reviewed with all parties involved in the study prior to its commencement.

Chemical Hazards

- ☆ The use of chemical hazards must be approved by the IACUC.
- ☆ During animal protocol submission [Appendix A](#) of the Protocol for Animal Care and Use must be completed and submitted to the Biosafety Committee for approval.
- ☆ Standard operating procedures for some agents are available on PINE under [IACUC](#).
- ☆ A discussion with the PBRC safety officer and the Director or Assistant Director of Comparative Biology prior to submission of protocols containing hazardous chemicals is recommended.

Radioisotopes

- ☆ The use of radioisotopes is prohibited in the CBC.
- ☆ For experiments requiring the use of radioactive materials in laboratory animals are needed contact LSU Radiation Safety Officer.
- ☆ IACUC approval is also required to perform terminal experiments using radioisotopes in animals.

Technical Resources

Personnel in the CBC are proficient in the performance of the techniques described/included in following sections. Research personnel are encouraged to seek training from CBC personnel to become proficient in all techniques necessary to complete their research protocols.

Drug Administration Guidelines for Rodents

Choice of needle size, injection site, and injection volume is critical for the successful administration of drugs and other compounds to rodents. The table below contains recommendations for safe and effective drug administration.

Species: Site Volume Needle	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous
Mouse	Intrascapular/scruff 2 – 3 ml 23, 25 G	Quadriceps/caudal thigh 0.05 ml 23, 25 G	Abdomen 2 – 3 ml 23, 25 G	Lateral tail vein 0.2 ml < 25 G
Rat	Intrascapular/scruff 5 - 10 ml 21, 23, 25 G	Quadriceps/caudal thigh 0.3 ml 23, 25 G	Abdomen 5 - 10 ml 23, 25 G	Lateral tail vein 0.5 ml < 23 G

Blood Collection in Guidelines for Rodents

Blood collection is one of the most common laboratory animal sampling techniques. The blood collection technique chosen should be based on consideration of a number of factors including:

- The species and size of the animal to be bled.
- The quantity (volume) and type (serum, plasma, whole cells) of sample required.
- The frequency of sampling.
- The health status and age of the animals to be bled.
- The training/experience of technician collecting the sample.

Survival sampling techniques commonly used in mice and rats are tail tip or lateral tail nick, retro-orbital sinus or plexus, and the saphenous vein. The quality and volume of sample necessary often dictates which technique is chosen. Blood volume collection guidelines are dependent on the circulating blood volume of the animal and the frequency of sampling. The circulating blood volume (BV) of rodents is estimated to be approximately 60 ml/kg body weight (60µl/10 g). General guidelines (see Table 1 below) recommend that no more than 10% of an animal's blood volume be removed at any one sampling every 2 – 4 weeks or 1% every 24h. Volumes outside of these guidelines require scientific justification and approval by the IACUC.

Table 1: Sample estimated blood volume sampling guidelines

Body Weight (g)	Estimated BV (ml)	1%	10%
20	1.2	0.012	0.12
30	1.8	0.018	0.18

40	2.4	0.024	0.24
100	6.0	0.06	0.6
200	12	0.12	1.2
300	18	0.18	1.8

Rodent Surgery Guidelines and Technical References

The NIH Office of Laboratory Animal Welfare has given the following guidance and requirements for performing rodent surgery. “The PHS Policy requires that the recommendations of the *Guide for the Care and Use of Laboratory Animals (Guide)* (NRC, 1985) be adhered to regarding survival surgical procedures and the environments in which they are conducted. The *Guide* standards for rodent are as follows: “..survival surgery may be conducted on rodents in an area that is used solely for this purpose while the surgery is being performed (such as a room or a portion of a room). The surgery must be performed using sterile instruments, surgical gloves, and aseptic procedures designed to prevent contamination of the operative site.” Other aspects of surgery are more thoroughly addressed in the *Guide* on pages 60 – 65.

Definitions:

- ☆ **Minor survival surgery:** any surgical procedure that does not expose a body cavity and causes little physical impairment (i.e. cannulation of a peripheral vessel, subcutaneous implantation of an osmotic minipump).
- ☆ **Major survival surgery:** any procedure that penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function (i.e. laparotomy, craniotomy, thoracotomy, joint replacement, limb amputation).
- ☆ **Non-survival surgery:** any surgical procedure in which the animal is euthanized prior to recovery from anesthesia.

Pre-operative planning:

Positive surgical outcomes are greatly influenced by proper planning. All phases of the surgical event should be thoroughly evaluated and planned to maximize the positive outcomes. Specific items to address are:

1. Location or physical environment – CBC surgery suites
2. Personnel responsibilities
3. Surgical supplies = surgical instruments, disposables, suture material, equipment needs
4. Drugs = anesthetics, analgesics, other drugs
5. Surgical records – see sample

Location:

All survival surgical procedures must be performed in the Comparative Biology Core surgical facilities (E1059, E1060, and E1061). The CBC surgery suites must be reserved in advance through the sign-up sheets available outside room E1027. All three surgical suites contain an isoflurane vaporizer, rodent induction boxes, water-jacketed heating pads, glass bead instrument sterilizers, and surgical lighting. CBC provides sterile general surgical packs, sterile gloves and gown packs; scrub tops, caps, and masks and surgical scrub for patient skin preparation. Principle investigators must supply suture material and scalpel blades. If specialized equipment is necessary in the surgical suites please pre-arrange these needs with Cindy Kloster.

Anesthesia:

The choice of an anesthetic regimen takes place during protocol preparation generally in consultation with the attending veterinarian. Anesthetic choice is based on species and age of animal, type and duration of operative procedure, and need for specialized intra-operative instrumentation (i.e. use of surgical microscope or stereotaxic device). Both injectable and inhalant anesthetic regimens are available for rodents (see [Anesthetic/Analgesic Infosheets](#)).

Stages of Anesthesia

Stage 1-Voluntary Excitement: Animals resist anesthesia, become frightened, struggle, causing an increased pulse, elevated blood pressure, and dilated pupils. Excessive salivation, voiding of urine and feces, and breath holding are common.

Stage 2- Involuntary Excitement: Involuntary movements followed by hypertonicity or rigidity of the skeletal muscles. Characterized by erratic respirations, a fast heart rate, elevated blood pressure, dilated pupils and rhythmic eye movements. All reflexes are present but slowed.

Stage 3- Surgical Anesthesia:

☆ *Plane 1- Light anesthesia:* Muscle tone diminishes and depression of the spinal cord results in relaxation of smaller muscles. Cough reflex is usually present. Pulse, blood pressure, and pupils return to normal. Eye movements are present in the beginning of this plane but disappear as the animal enters plane 2. **This stage is not adequate for most surgery.**

☆ *Plane 2- Moderate Surgical Anesthesia.* Temperature regulating, cough, and vomiting centers are depressed. Respiration is regular and deep. The pulse rate and blood pressure are essentially normal. The eyes are fixed. Pharyngeal, laryngeal, and peritoneal reflexes disappear. Eyelid and pedal reflexes disappear in the latter part of this plane. Corneal reflex may persist into the upper part of Plane 3. **Most surgeries can be performed in this plane. A good level is one at which the pedal reflex is lost and the corneal reflex is just present.**

☆ *Plane 3- Deep Anesthesia:* Muscle tone tends to decrease and the animal becomes more depressed and thoracic movement tends to lag and abdominal movements evident to progressive intercostal paralysis.

☆ *Plane 4- profound anesthesia:* The pulse becomes rapid and weak, blood pressure tends to decline, and respiration is shallow and purely abdominal due to commencing paralysis. The animal is completely flaccid and the anal sphincter reflex disappears. The pupils dilate.

Stage 4- Bulbar paralysis: The medullary centers are paralyzed, respiration stops, blood pressure falls to shock levels, and the heart gradually fails. Death.

Animal preparation:

1. Induce anesthesia.
2. Surgical prep should take place in a site separate from where the operative procedure will be performed.
3. First clip hair from the surgical site.
4. Next, the site should be scrubbed with 3 alternating washes of Nolvasan surgical scrub followed by 70% alcohol wipes (e.g. Nolvasan #1 alcohol #1; Nolvasan #2 alcohol #2; Nolvasan #3 alcohol #3).
5. Care must be taken not to over-wet the patient during surgical prep due to the risk of hypothermia.
6. Once the patient is prepared it can be moved to the surgical area and positioned for surgery.

Surgeon preparation:

1. The surgeon should wear a surgical mask, cap, clean lab coat or scrub top, and sterile surgical gloves.
2. Prior to donning sterile surgical gloves the surgeon should wash his/her hands with an antiseptic surgical scrub located in room E1026.
3. Others assisting in surgery should likewise wear a surgical mask and clean lab coat or scrub top and gloves when handling the animals.

Surgery:

1. Assess that the animal is at a **surgical plane of anesthesia** by monitoring toe pinch reflex and respiratory characteristics
 - a. Animals in a surgical plane of anesthesia should be non-responsive to a toe pinch and respirations should be smooth and regular (See “Stages of Anesthesia”).
 - b. Anesthesia monitoring of the patient must be continued throughout the procedure (i.e. perform toe pinch every 10 minutes)
 - c. Often an increase in respiratory rate is noted prior to an animal becoming responsive to the toe pinch.
 - d. If animal becomes responsive administer additional anesthetic (injectable protocols) or increase vaporizer setting (isoflurane).
2. A **sterile instrument pack** should be opened at the beginning of surgery and aseptic technique must be employed throughout surgery.
 - a. If multiple animals are operated at one sitting instruments may be sterilized between animals by the use of a glass bead sterilizer.
 - b. Proper use of the glass bead sterilizer is important for asepsis and life of surgical instruments.
 - c. The sterilizer should be turned on prior to starting surgery and allowed to come to operating temperature (250°), which may require 15 minutes.
 - d. The surgeon should have a small basin of sterile water available for removal of any blood or tissue fluids from the instruments.
 - e. Wipe off instruments to be sterilized with a sterile water-soaked gauze.
 - f. Place in preheated glass bead sterilizer for 30 seconds.
 - g. Allow instrument to cool completely prior to using on animal.
3. **Tissue handling**
 - a. Surgeons must be mindful the gentle tissue handling is critical for the prevention of post-operative complications and rapid wound healing.
 - b. Use of appropriately sized “rat-toothed” forceps is the least traumatic method of handling tissues.

Wound closure:

1. Choice of wound closure materials and techniques are made during the preparation of the animal use protocol.
2. Absorbable suture materials (i.e. gut, Vicryl) are used whenever the suture material will be “buried” (i.e. abdominal muscle wall, subcutaneous tissues).
3. Non-absorbable sutures (i.e. silk, nylon) and wound clips are appropriate for skin closure.
4. The absorbable suture material, Vicryl, may also be used for skin closure.
5. Although silk suture has been used for many years in rodent surgery it is considered to be very irritating and should never be placed in a “buried” location.

Animal recovery:

1. In the immediate post-operative period the animal should be placed in a warm, quiet environment for recovery.
2. In cases of prolonged surgical procedures or substantial blood loss providing warmed subcutaneous fluids (Lactated Ringers solution, 0.9% saline, or 5% dextrose) can improve survival and speed recovery from anesthesia (See Postoperative Fluid Therapy in Rodents on page 34).
3. Animals must be monitored frequently (every 15 minutes) until recovered from anesthesia and able to ambulate.
4. Once recovered the animal may be returned to its normal housing.

Post-operative care:

1. Post-operative care is the responsibility of the investigative staff.
2. Animals should be monitored at least daily for 7 days post-operatively specifically noting the incision site, animal demeanor, and urine/feces production as a measure of food and water intake.
3. The investigative team should maintain records documenting care and observations during the post-operative period (See [Appendix H](#) - Rodent Post Operative Record Sheet).

Analgesics:

1. Administration and documentation of the use of analgesics is the responsibility of the investigative staff.
2. Daily post-operative assessment should include indications that the analgesic regimen is adequate for each animal.
3. Consult the CBC veterinary staff for animals that appear to need additional analgesics.

Suture removal:

1. Removal of skin sutures or wound clips is the responsibility of the principle investigator.
2. Generally removal can be safely accomplished 7 – 14 days post-operatively.
3. For assistance with suture removal contact the CBC veterinary staff.

Complications

In the event of intra-operative or post-operative complications (slow recovery, problems with wound healing, infection) contact the CBC veterinary staff.

Anesthetic and Analgesic Formulary

A number of drugs and drug combinations are available for use as anesthetics and analgesics in rodents. The proper use of anesthetics and analgesics is an ethical, veterinary medical, and scientific requirement. The selection of the most appropriate anesthetic and analgesic protocol requires experience and professional judgment. The regimen chosen must adequately meet the clinical and humane requirements without compromising the scientific aspects of the study. All investigators are encouraged to seek the advice of the attending veterinarian during the preparation of a Protocol for Animal Care and Use so that the most appropriate agents can be included for anesthesia and analgesia. The following tables contain the anesthetic and analgesic agents available in Comparative Biology for use in rodents. Infosheets for these agents follow with a more complete description of their recommended use.

Anesthetic Agents

Agent	Species	Dose	Route	Frequency	Controlled Substance
Isoflurane	M, R	1.5 – 3%	Inhalation	Too effect	No
Rodent Cocktail [®]	M, R	See R/C SOP	SC	As needed	Yes
Ketamine/xylazine	M, R	See K/X SOP	SC, IP	As needed	Yes
Ketamine/ medetomidine	M, R	See K/M SOP	SC, IP	As needed	Yes
Nembutal	M, R	~ 50mg/kg	IP	As needed	Yes

@ Ketamine/acepromazine/xylazine combination

Remember: procedures that are painful in humans must be considered painful in animals and analgesics must be provided to alleviate that pain unless withholding of analgesics is scientifically justified and approved by the IACUC.

Analgesic Agents

Analgesic Agent	Species	Dose	Route	Frequency	Controlled Substance
Buprenorphine	Mice	0.05 – 0.1 mg/kg	SC	Q 12 h	Yes
Buprenorphine	Rats	0.01 – 0.05 mg/kg	SC or IV	Q 8 – 12 h	Yes
Carprofen	Mice and rats	5 mg/kg	SC	Q 24 h	No
Bupivacaine/ lidocaine	Mice and rats	Max = 4 mg/kg	Incisional	Once at time of surgery.	No
Ibuprofen [*]	Mice	40 mg/kg	PO in drinking water	Once daily	No
Ibuprofen [#]	Rats	15 mg/kg	PO in drinking water	Once daily	No
Tylenol [@]	Mice and rats	300 mg/kg	PO in drinking water	Once daily	No

* Ibuprofen for **mice**: Mix 4.7 ml of Children's Motrin (20mg/ml) in 500 ml of water.

Ibuprofen for **rats**: Mix 2.35 ml of Children's Motrin (20mg/ml) in 500 ml of water.

@ Tylenol: Mix 24 ml of Children's Tylenol (32 mg/ml) in 500 ml of water.

The following infosheets were prepared for investigators to help clarify the various anesthetic and analgesic drugs available in the CBC for use in rodents.

Rodent Cocktail

1. Purpose: To describe the preparation and use of rodent cocktail for rodent anesthesia.
2. Definitions:
 - 2.1. Rodent cocktail is a mixture of ketamine/acepromazine/xylazine used as an injectable anesthetic for rats and mice.
 - 2.2. Ketamine (a controlled substance) is an injectable dissociative anesthetic.
 - 2.3. Acepromazine is a phenothiazine sedative.
 - 2.4. Xylazine is an α -2-adrenergic agonist tranquilizer.
3. Preparation:
 - 3.1. To a 10 ml bottle of ketamine (100mg/ml) add 2 mls of acepromazine (10 mg/ml) and 0.5 ml of xylazine (100mg/ml).
 - 3.2. Final drug concentrations are 80 mg/ml ketamine, 1.6 mg/ml acepromazine, and 4 mg/ml xylazine.
 - 3.3. This cocktail is available from CBC – see Cindy Kloster.
4. Preanesthesia with atropine:
 - 4.1. Atropine is an anticholinergic that decreases bronchial secretions and protects the heart from vagal inhibition.
 - 4.2. A preanesthetic dose of atropine may be given 15 minutes prior to administration of rodent cocktail.
 - 4.3. The preanesthetic dose of atropine is 0.05mg/kg.
 - 4.4. To use atropine dilute 0.5 ml in 4.5 ml of normal saline then administer subcutaneously at a rate of 0.1 ml/100g body weight.
5. Anesthesia with rodent cocktail:
 - 5.1. Administer subcutaneously at a dose rate of 1.25 ml/kg or 0.125-ml/100 g of body weight.
 - 5.2. This dose corresponds to a ketamine dose of 100 mg/kg, an acepromazine dose of 2mg/kg, and a xylazine dose of 5 mg/kg.
 - 5.3. After administration the animal should reach a surgical plane of anesthesia within 10 – 15 minutes.
 - 5.4. This plane of anesthesia will generally be maintained for approximately 20 minutes.
6. Supplemental anesthesia:
 - 6.1. For procedures lengthy surgical procedures supplemental anesthetic doses will need to be administered.
 - 6.2. For supplemental doses use ketamine only at a dose rate of 80 mg/kg or 8 mg/100 g body weight. This can be achieved by administering 0.08 ml of ketamine/100 g body weight.

Ketamine/Xylazine Rodent Anesthesia

1. Purpose: To describe the preparation and use of ketamine/xylazine rodent anesthetic.
2. Definitions:
 - 2.1. Ketamine/xylazine mixture may be used as an injectable anesthetic for rats and mice.
 - 2.2. Ketamine (a controlled substance) is an injectable dissociative anesthetic.
 - 2.3. Xylazine is a α -2-adrenergic agonist tranquilizer.
3. Preparation of stock solution:
 - 3.1. To a 10 ml bottle of ketamine (100mg/ml) add 1.0 ml of xylazine (100mg/ml).
 - 3.2. Final drug concentrations are 90 mg/ml ketamine and 9 mg/ml xylazine.
 - 3.3. This cocktail is available from CBC – see Cindy Kloster.
4. Preanesthesia with atropine:
 - 4.1. Atropine is an anticholinergic that decreases bronchial secretions and protects the heart from vagal inhibition.
 - 4.2. A preanesthetic dose of atropine may be given 15 minutes prior to administration of rodent cocktail.
 - 4.3. The preanesthetic dose of atropine is 0.05mg/kg.
 - 4.4. To use atropine dilute 0.5 ml in 4.5 ml of normal saline then administer subcutaneously at a rate of 0.1 ml/100g body weight.
5. Anesthesia with K/X combination:
 - 5.1. Rats
 - 5.1.1. Administer stock solution IP or IM at a dose rate of 1ml/kg or 0.1ml/100 g of body weight.
 - 5.1.2. This dose corresponds to a ketamine dose of 90 mg/kg and a xylazine dose of 9 mg/kg.
 - 5.1.3. After administration the animal should reach a surgical plane of anesthesia within 10 – 15 minutes.
 - 5.1.4. This plane of anesthesia will generally be maintained for approximately 20 minutes.
 - 5.2. Mice:
 - 5.2.1. Dilute stock solution 1:5 (1 part stock : 4 parts sterile water for injection).
 - 5.2.2. Administer at a dose rate of 0.1 ml/20 g or body weight.
 - 5.2.3. This dose corresponds to a ketamine dose of 90 mg/kg and a xylazine dose of 9 mg/kg.
 - 5.2.4. After administration the animal should reach a surgical plane of anesthesia within 10 – 15 minutes.
 - 5.2.5. This plane of anesthesia will generally be maintained for approximately 20 minutes.
6. Supplemental anesthesia
 - 6.1. For lengthy surgical procedures supplemental anesthetic doses will need to be administered.
 - 6.2. For supplemental doses use give $\frac{1}{4}$ of the original total dose.

Ketamine/Medetomidine Rodent Anesthesia

1. Purpose: To describe the preparation and use of ketamine/medetomidine rodent anesthetic.
2. Definitions:
 - 2.1. Ketamine/medetomidine mixture may be used as an injectable anesthetic for rats and mice.
 - 2.2. Ketamine (a controlled substance) is an injectable dissociative anesthetic.
 - 2.3. Medetomidine is an α -2-adrenergic agonist tranquilizer.
 - 2.4. Atipamezole is an α -2-adrenergic antagonist used as a reversal agent for medetomidine.
3. Preparation
 - 3.1. Rats
 - 3.1.1. Mix 0.75 ml of ketamine (100mg/ml) with 0.5 ml of medetomidine (1 mg/ml).
 - 3.1.2. Add 0.75 ml of sterile water for injection; this will give final drug concentrations of 37.5 mg/ml ketamine and 0.25 mg/ml medetomidine.
 - 3.1.3. This cocktail is available from CBC – see Cindy Kloster.
 - 3.2. Mice
 - 3.2.1. Mix 0.38 ml of ketamine (100mg/ml) with 0.5 ml of medetomidine (1 mg/ml).
 - 3.2.2. Add 4.12 ml of sterile water for injection; this will give final drug concentrations of 7.6 mg/ml ketamine and 0.5 mg/ml medetomidine.
 - 3.2.3. This cocktail is available from CBC – see Cindy Kloster.
4. Preanesthesia with atropine
 - 4.1. Atropine is an anticholinergic that decreases bronchial secretions and protects the heart from vagal inhibition.
 - 4.2. A preanesthetic dose of atropine may be given 15 minutes prior to administration of rodent cocktail.
 - 4.3. The preanesthetic dose of atropine is 0.05mg/kg.
 - 4.4. To use atropine dilute 0.5 ml in 4.5 ml of normal saline then administer subcutaneously at a rate of 0.1 ml/100g body weight.
5. Anesthesia with K/M combination
 - 5.1. Rats
 - 5.1.1. Administer IP at a dose rate of 0.2ml/100 g of body weight.
 - 5.1.2. This dose corresponds to a ketamine dose of 75 mg/kg and a medetomidine dose of 0.5 mg/kg.
 - 5.1.3. After administration the animal should reach a surgical plane of anesthesia within 10 – 15 minutes.
 - 5.1.4. This plane of anesthesia will generally be maintained for approximately 20 minutes.
 - 5.2. Mice
 - 5.2.1. Administer at a dose rate of 0.1 ml/10 g or body weight.
 - 5.2.2. This dose corresponds to a ketamine dose of 75 mg/kg and a medetomidine dose of 1 mg/kg.
 - 5.2.3. After administration the animal should reach a surgical plane of anesthesia within 10 – 15 minutes.
 - 5.2.4. This plane of anesthesia will generally be maintained for approximately 20 minutes.
6. Supplemental anesthesia
 - 6.1. For procedures lengthy surgical procedures supplemental anesthetic doses will need to be administered.
 - 6.2. For supplemental doses use give $\frac{1}{4}$ of the original total dose.
7. Reversal
 - 7.1. At the end of a surgical procedure the sedative effect of medetomidine can be reversed using the reversal agent atipamezole.

- 7.2. The dose of atipamezole is 1 mg/kg for rats and mice.
- 7.3. For rats mix 0.2 ml of atipamezole with 0.8 ml of sterile water for injection and administer at a rate of 0.1 ml/100g body weight.
- 7.4. For mice mix 0.1 ml of atipamezole with 4.9 ml of sterile water for injection and administer at a rate of 0.1 ml/10 g body weight.

Use of Bupivacaine/Lidocaine Local Anesthesia

1. Purpose: To describe the preparation and use of bupivacaine/lidocaine for local anesthesia in rodents.
2. Definitions:
 - 2.1. Bupivacaine is a longer acting local anesthetic.
 - 2.2. Lidocaine is a shorter acting local anesthetic.
3. Rationale:
 - 3.1. Giving bupivacaine/lidocaine pre-operatively at the surgical site will decrease the amount of general anesthetic necessary.
 - 3.2. Giving bupivacaine/lidocaine pre-operatively at the surgical site will decrease post-operative pain by minimizing stimulation of pain fibers during surgical manipulation thereby decreasing the amount of post-operative analgesics needed.
 - 3.3. The duration of action for bupivacaine is approximately 6 h therefore affording considerable post-operative analgesia.
4. Preparation:
 - 4.1. Bupivacaine and lidocaine are mixed at a 1:1 ratio.
 - 4.2. This mixture is available from CBC – see Cindy Kloster.
5. Local anesthesia during surgery with bupivacaine/lidocaine:
 - 5.1. The dose rate is 2.5 – 12.5 mg/kg.
 - 5.2. Administer between 0.2 – 1 ml for a 200 – 300g rat.
 - 5.3. For a 250 g **rat** 0.2 – 0.4 ml will adequately infuse the surgical site.
 - 5.4. For a 25 g **mouse** use 0.05 – 0.1 ml of mixture to infuse surgical site.
 - 5.5. Once general anesthesia is induced, infuse B/L mixture subcutaneously at the incision site using a 1ml syringe fitted with a 25 ga needle.
 - 5.6. After administration allow 2 – 3 minutes before starting surgery or immediately into incision just prior to suturing.
6. Local anesthesia with bupivacaine/lidocaine after tail tip collection when genotyping or after blood collection:
 - 6.1. Place aliquot of b/l mixture in small receptacle (i.e. eppendorf tube).
 - 6.2. After excising tail tip, dip tip of tail briefly (5 – 10 sec) in b/l mixture or a cotton swab dipped in the b/l mixture can be held on the excised tail tip briefly.
 - 6.3. Cauterize with silver nitrate.
 - 6.4. Place animal back in cage.

Use of Buprenorphine for Analgesia in Rats

1. Purpose: To describe the preparation and use of buprenorphine as a postoperative analgesic in rats.
2. Definitions:
 - 2.1. Buprenorphine is a controlled substance requiring careful record keeping – see Cindy Kloster for instructions.
 - 2.2. Buprenorphine is a potent partial mu agonist that has a prolonged duration of action in many species.
 - 2.3. Injectable buprenorphine is supplied at 0.3 mg/ml.
3. Rationale:
 - 3.1. Giving buprenorphine pre-operatively will decrease the amount of post-op analgesic necessary.
 - 3.2. The duration of action for buprenorphine is approximately 6-8 h.
 - 3.3. The analgesic regimen for each surgical procedure may be different and should be found in Section 9.6 of the PBRC Protocol for Animal Care and Use for the particular study the animals are being used under.
4. Preparation:
 - 4.1. Comparative Biology dilutes buprenorphine 1:10 to provide a final concentration as supplied to the investigator of 0.03 mg/ml.
 - 4.2. This diluted drug is available from CB – see Cindy Kloster.
5. Post-operative analgesia with buprenorphine:
 - 5.1. The dose rate is **0.01 – 0.05 mg/kg**.
 - 5.2. Administer 0.1 – 0.4 ml of the 0.03mg/ml dilution subcutaneously for each 250 g of body weight.**
 - 5.3. Once general anesthesia is initiated administer the initial dose of buprenorphine subcutaneously a 1ml syringe fitted with a 25-ga needle.
 - 5.4. For most surgical procedures a second dose should be administered 6 – 8 h later.
 - 5.5. Animals should be minimally monitored once a day post-operatively to check for signs of pain or discomfort and additional doses of analgesics given if necessary.
 - 5.6. For questions concerning the post-operative pain monitoring consult with Dr. Robert or Cindy Kloster.

Use of Buprenorphine for Analgesia in Mice

1. Purpose: To describe the preparation and use of buprenorphine as a postoperative analgesic in mice.
2. Definitions:
 - 2.1. Buprenorphine is a controlled substance requiring careful record keeping – see Cindy Kloster for instructions.
 - 2.2. Buprenorphine is a potent partial mu agonist that has a prolonged duration of action in many species.
 - 2.3. Injectable buprenorphine is supplied at 0.3 mg/ml.
3. Rationale:
 - 3.1. Giving buprenorphine pre-operatively will decrease the amount of post-op analgesic necessary.
 - 3.2. The duration of action for buprenorphine is approximately 12 h.
 - 3.3. The analgesic regimen for each surgical procedure may be different and should be found in Section 9.6 of the PBRC Protocol for Animal Care and Use for the particular study the animals are being used under.
4. Preparation (done by Comparative Biology personnel):
 - 4.1. Comparative Biology dilutes buprenorphine 1:30 to provide a final concentration as supplied to the investigator of 0.01 mg/ml.
 - 4.2. This diluted drug is available from CB – see Cindy Kloster.
5. Post-operative analgesia with buprenorphine:
 - 5.1. The dose rate is **0.05 – 0.1 mg/kg**.
 - 5.2. Administer 0.1 – 0.2 ml of the 0.01mg/ml dilution subcutaneously for each 20 g of body weight.**
 - 5.3. Once general anesthesia is initiated administer the initial dose of buprenorphine subcutaneously a 1ml syringe fitted with a 25-ga needle.
 - 5.4. For most surgical procedures a second dose should be administered 12 h later.
 - 5.5. Animals should be minimally monitored once a day post-operatively to check for signs of pain or discomfort and additional doses of analgesics given if necessary.
 - 5.6. For questions concerning the post-operative pain monitoring consult with Dr. Robert or Cindy Kloster.

Use of Carprofen for Analgesia in Rats

1. Purpose: To describe the preparation and use of carprofen as a postoperative analgesic in rats.
2. Definitions:
 - 2.1. Carprofen is a nonsteroidal anti-inflammatory veterinary drug.
 - 2.2. Injectable carprofen is supplied at 50 mg/ml.
3. Rationale:
 - 3.1. Giving carprofen pre-operatively will decrease the amount of post-op analgesic necessary.
 - 3.2. The duration of action for carprofen is approximately 24 h therefore affording considerable post-operative analgesia.
 - 3.3. The analgesic regimen for each surgical procedure may be different and should be found in Section 9.6 of the PBRC Protocol for Animal Care and Use for the particular study the animals are being used under.
4. Preparation:
 - 4.1. Comparative Biology dilutes carprofen 1:10 to provide a final concentration as supplied to the investigator of 5 mg/ml.
 - 4.2. **Diluted carprofen must be refrigerated.**
 - 4.3. This diluted drug is available from CB – see Cindy Kloster.
5. Post-operative analgesia with carprofen:
 - 5.1. The dose rate is **5 mg/kg**.
 - 5.2. **Administer 0.1 ml of the 5mg/ml dilution subcutaneously for each 100 g of body weight.**
 - 5.3. For a 250 g **rat** administer 0.25 ml of the diluted carprofen.
 - 5.4. Once general anesthesia administer the initial dose of carprofen subcutaneously a 1ml syringe fitted with a 25 ga needle.
 - 5.5. For most surgical procedures a second dose should be administered 24 h later.
 - 5.6. Animals should be monitored minimally once daily post-operatively to check for signs of pain or discomfort and additional doses of carprofen given once daily if necessary.
 - 5.7. Other nonsteroidal anti-inflammatory drugs such as Tylenol and ibuprofen should not be used in conjunction with carprofen.
 - 5.8. For questions concerning the post-operative pain monitoring consult with Dr. Robert or Cindy Kloster.

Use of Carprofen for Analgesia in Mice

1. Purpose: To describe the preparation and use of carprofen as a postoperative analgesic in mice.
2. Definitions:
 - 2.1. Carprofen is a nonsteroidal anti-inflammatory veterinary drug.
 - 2.2. Injectable carprofen is supplied at 50 mg/ml.
3. Rationale:
 - 3.1. Giving carprofen pre-operatively will decrease the amount of post-op analgesic necessary.
 - 3.2. The duration of action for carprofen is approximately 24 h therefore affording considerable post-operative analgesia.
4. Preparation:
 - 4.1. Comparative Biology dilutes carprofen 1:100 to provide a final concentration as supplied to the investigator of 0.5 mg/ml.
 - 4.2. **Diluted carprofen must be refrigerated.**
 - 4.3. This diluted drug is available from CB – see Cindy Kloster.
5. Post-operative analgesia with carprofen:
 - 5.1. The dose rate is **5 - 10 mg/kg**.
 - 5.2. **Administer 0.1 – 0.2 ml of the 0.5mg/ml dilution subcutaneously for each 10 g of body weight.**
 - 5.3. For a 25 g **mouse** administer 0.25 – 0.5 ml of the diluted carprofen.
 - 5.4. Once general anesthesia administer the initial dose of carprofen subcutaneously a 1ml syringe fitted with a 25 ga needle.
 - 5.5. For most surgical procedures a second dose should be administered 24 h later.
 - 5.6. Animals should be minimally monitored once a day post-operatively to check for signs of pain or discomfort and additional doses of carprofen given once daily if necessary.
 - 5.7. Other nonsteroidal anti-inflammatory drugs such as Tylenol and ibuprofen should not be used in conjunction with carprofen.
 - 5.8. For questions concerning the post-operative pain monitoring consult with Dr. Robert or Cindy Kloster.

Postoperative Fluid Therapy in Rodents

1. Purpose: To describe the use of parenterally-administered fluids postoperatively in mice and rats.
2. Definitions:
 - 2.1. Parenteral administration of fluids refers to the injection of replacement fluids either intravenously, subcutaneously, or intraperitoneally.
 - 2.2. Fluids for parenteral administration kept in the CBC surgical area include, lactated ringers solution, 0.9% sodium chloride, and 5% dextrose.
3. Rationale:
 - 3.1. Giving parenteral fluids immediately post-operatively will provide fluids to replace any lost during surgery and decrease the likelihood of the patient becoming dehydrated in the post-operative period.
 - 3.2. Administration of fluids may also help to speed recovery from anesthesia.
4. Preparation:
 - 4.1. Comparative Biology maintains fluids in an incubator at 37° C in E1026 for use in rodents post-operatively.
5. Post-operative fluid therapy recommendations:
 - 5.1. For most species maintenance fluid requirements are approximately 40 – 80 ml/kg/24h.
 - 5.2. For a 30g mouse administer 1 – 2 ml of warmed fluids subcutaneously or intraperitoneally prior to placing animal in recovery chamber.
 - 5.3. For a 250 g rat administer 5 – 6 ml of warmed fluids subcutaneously or intraperitoneally prior to placing animal in recovery chamber.
 - 5.4. Twenty-four hours after surgery observe the animal for signs of dehydration (loss of skin elasticity, sunken/dull eyes) and urine/fecal production. If the animal appears dehydrated or has not produced adequate urine/feces administer fluids again.
 - 5.5. For additional questions concerning the post-operative fluid therapy consult with Dr. Robert or Cindy Kloster.

Appendices

<u>Appendix A</u>	<u>CBC Procedure Request Form</u>
<u>Appendix B</u>	<u>Organizational Chart</u>
<u>Appendix C</u>	<u>IACUC Roster</u>
<u>Appendix D</u>	<u>Sample Protocol Amendment</u>
<u>Appendix E</u>	<u>Zoonotic Disease and Laboratory Animal Allergy</u>
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Appendix D: Sample Protocol Amendment

Memorandum

To: Dr. Richard Rogers, IACUC Chair

From: Dr. John Smith

Date: September 28, 2005

Re: Amendment to protocol # XXXX

I would like to request the following change to my above referenced protocol. In our protocol we had time points of 2, 4, and 6 weeks. Results from our initial experiments were encouraging however did not reach statistical significance. In our next set of experiments we would like to add two additional time points of 8 and 12 weeks. We will substitute the animals from the original 2- and 4-week time points therefore no additional animals will be necessary to complete the experiments. All other procedures are as described in the original protocol however the time frames will be shifted to these later time points.

Appendix E: Zoonotic Disease and Laboratory Animal Allergy

Zoonotic diseases are diseases of animals that may be transmitted to humans. Rodents-borne zoonotic diseases are rare when rodents are obtained from reliable vendors and therefore are uncommon in modern animal facilities. Colony-born laboratory rodents are generally docile, but may occasionally inflict injury from a bite or scratch. Although rodents may carry organisms that may be potentially infectious to humans; the major health risk to individuals working with laboratory rodents is the development of an allergy. The development of disease in the human host often requires a preexisting state that compromises the immune system. If you have an immune-compromising medical condition or you are taking medications that impair your immune system (steroids, immunosuppressive drugs, or chemotherapy) you are at higher risk for contracting a rodent disease and should consult your physician. The following is a partial list of potential rodent zoonoses.

Lymphocytic choriomeningitis: Lymphocytic choriomeningitis (LCM) is caused by the arenavirus commonly associated with hamsters, but does infect mice and is quite common in the wild mouse population. Infection in the mouse is usually asymptomatic. LCM is rare in laboratory animal facilities. We do test our sentinels for LCM. Transmission to humans is through contact with infected tissues including tumors, feces, urine, or aerosolization of any one of these. Disease in humans is generally flu-like symptoms including fever, myalgia, headache, and malaise.

Leptospirosis: *Leptospira spp.* most commonly associated with livestock and dogs but has also been isolated from wild rodents. Leptospire are in the urine of infected animals and are transmitted through direct contact with urine or tissues of infected animals or via skin abrasions or contact with mucous membranes. Transmission can also occur through inhalation of infectious droplet aerosols and by ingestion. The disease in people can vary from mild to severe. In severe cases the disease may become multi-systemic with chronic sequelae. Initial symptoms include fever, weakness, headaches, and myalgia.

Hantavirus Infection: Hantaviruses occurs commonly in wild rodent populations. Rats and mice have been implicated in outbreaks of the disease. Hantavirus infections in humans have occurred from exposure to laboratory rats in Japan, Korea, Belgium, France and England. In the US hantavirus infections has been associated with wild rodent exposure. Rodents shed the virus in their respiratory secretions, saliva, urine and feces. Transmission to humans is via inhalation of infectious aerosols. The clinical signs are related to the species of hantavirus involved.

***Streptobacillus moniliformis* and *Spirillum minus*:** These bacteria cause a disease known as “rat bite fever”. These organisms are present in the mouth and upper respiratory tract of asymptomatic rats. Transmission is believed to be from the bite of an infected rat. The incubation period is from 1 – 10 days. The disease in humans is characterized by fever, lymphadenopathy, myalgia, headache, malaise and may be accompanied by a rash. Most cases spontaneously resolve in 14 days however some cases become multisystemic and may be fatal.

Other Diseases: There are several other (less common) diseases that may be spread by working with laboratory rodents. These include dematophytosis (fungal infection, ringworm), poxviruses of rats, *Streptococcus pneumoniae*, and *Hymenolepis nana*.

Laboratory animal allergies

By far the greatest occupational risk to working with rodents is the development of laboratory animal allergy. Workers with pre-existing allergies are at greater risk. Studies indicate that as many as 50% of laboratory animal workers are affected by laboratory animal allergies. Animal dander, hair, saliva, feces, and urine in particular, contain powerful allergens that can cause both contact skin allergies and/or respiratory symptoms.

How to protect yourself from zoonotic diseases and laboratory animal allergies

- **Wear gloves.** When working with rodents wear appropriate gloves for the task and wash your hands after removing gloves.
- **Wear respiratory protection.** Masks should be worn to minimize the risk of aerosol transmission of zoonotic agents and to minimize the exposure to laboratory animal allergens. Fit testing of NIOSH N95 masks is recommended and can be done by the PBRC safety officer.
- **Wear other protective clothing.** Lab coats are available in Comparative Biology and should be worn when working with

rodents. The lab coats should be left in the CBC when they will be laundered.

- **Wash your hands.** One of the single most effective preventative measure that can be taken is thorough, regular hand washing. Wash hands and arms after handling any animal. Never smoke, drink or eat in the Comparative Biology animal rooms or before washing your hands when you leave the CBC.
- **Seek Medical Attention Promptly.** If you are injured in the CBC, promptly report the accident to the assistant director, even if it seems relatively minor. Minor cuts and abrasions should be immediately and thoroughly cleansed with antibacterial soap and then protected from further exposure to rats and mice.
- **Tell your physician you work with rodents.** Whenever you are ill, even if you're not certain that the illness is work-related, always mention to your physician that you work with rodents. Many zoonotic diseases have flu-like symptoms and would not normally be suspected. Your physician needs this information to make an accurate diagnosis. Questions regarding personal human health should be answered by your physician.

Appendix F: Rodent Disease Chart

Agent	Species Affected	Ages Affected	Transmission	Tissues Affected	Clinical Disease	Duration of Infection
Mouse Parvovirus (Parvovirus)	Mice	All	Orofecal, Respiratory	Lymphoid	None	Chronic, persistent
Epizootic Diarrhea of Infant Mice (Rotavirus)	Mice	Neonates	Orofecal	Gastrointestinal Tract	Diarrhea	Acute
Mouse Hepatitis Virus (Coronavirus)	Mice	All	Orofecal	Numerous	Subclinical to lethal	Acute
Minute Virus of Mice (Parvovirus)	Mice	All	Contact	CNS, Kidney	Usually subclinical	Chronic, latent
Ectromelia (Orthopoxvirus)	Mice	All	Respiratory, Orofecal, Urine, Direct Contact.	Numerous	Skin lesion or acute death.	Acute
Sendai Virus (Paramyxovirus)	Mice/Rats	All	Respiratory	Respiratory Tract	Tracheobronchitis – pneumonia	Acute
Pneumonia Virus of Mice (Paramyxovirus)	Mice/Rats	All	Respiratory	Lower Respiratory Tract	Pneumonia	Acute
Reovirus 3 (Reovirus)	Mice/Rats	Usually neonates	Orofecal	Multiple	Diarrhea	Acute
Theiler's Murine Encephalomyelitis Virus (Picornavirus)	Mice/Rats	Adult	Orofecal	CNS	Convulsion, paralysis	Variable
<i>Mycoplasma pulmonis</i>	Mice/Rats	All	Respiratory	Respiratory	Pneumonia	Chronic
Lymphocytic Choriomeningitis Virus	Mice/Rats	All	In utero as well as numerous others possible.	Numerous	Subclinical to mild. Note this is a zoonotic disease.	Acute to chronic.
Pinworms	Mice/Rats	All	Orofecal	Gastrointestinal Tract	None to mild.	Chronic
Rat Parvovirus (Parvovirus)	Rats	All	Oronasal	Numerous	Subclinical to lethal	Acute, persistent?
Rat Coronavirus, Sialodacryadenitis Virus (Coronavirus)	Rats	All	Respiratory	Salivary Glands, Harderian Glands, Respiratory Tract	Mild to severe	Acute

Appendix L: Animal Housing Density

The table below includes the recommendations of the *Guide* for the housing density of mice and rats based on in²/g body weight. Comparative Biology recommends that animals be weaned at the maximum adult animal housing density. When are housed at a greater density they quickly attain their body weight become overcrowded.

Recommended Space for Commonly Used Group-Housed Laboratory Rodents

Animals	Weight, g	Floor Area/Animal, in ² _a	Height, in ^c	Std cage, in ² : # of adults Mouse	Std cage, in ² : # of adults Lg mouse or rat
Mice	<10	6	5		
	Up to 15	8	5		
	Up to 25	12	5		
Adult wt	>25 ^d	>15	5	68 : 4	150 : 9
Rats	<100	17	7		
	Up to 200	23	7		
	Up to 300	29	7		
	Up to 400	40	7		
Adult wt	Up to 500	60	7		142 : 2
	>500 ^d	>70	7		