A. What is the Georgia State University IACUC?

1. IACUC Oversight ................................................................. 7
2. Principal Investigator Responsibilities .................................. 10

B. Required Educational Program on Animal Care and Use (TRAINING) .... 14

1. Facility Access ............................................................... 15
2. Online Training ............................................................. 15
   a. Required Modules ....................................................... 15
   b. Training Module Completion .................................... 15

C. Occupational Health and Safety Program Related to Animal Care and Use at Georgia State University ................................................................. 18

D. Protocol Review and Approval ............................................... 18

1. Submission and Processing of Animal Use Applications ............... 22
2. IACUC Meetings ............................................................ 22
3. Duration of Protocol Approval .......................................... 22
4. New Protocol Submission ................................................ 22
5. Protocol Amendment Submission ..................................... 23
6. Administrative Review and Approval .................................. 23
7. Notification of Protocol Status .......................................... 25
8. Post-Approval Monitoring ............................................... 26
9. Three year review of Protocols ......................................... 28
10. Suspension of Research Activity ...................................... 29
11. Protocol Flowchart ....................................................... 30
12. Amendment Flowchart .................................................. 31

E. Completing the IACUC Protocol Form .................................... 32

1. Regulatory Criteria .......................................................... 32
2. Why the Use of Animals in Research is Important ....................... 33
3. Describing Your Animal Studies ....................................... 33
4. Species Selection ........................................................... 34
5. Species Justification ....................................................... 34
6. Animal Numbers Justification ............................................. 34
7. Reduction, Replacement, Refinement .................................... 35
8. Search for Animal Alternatives .......................................... 36
9. Unnecessary Duplication .................................................. 38
10. Pain and Distress Categories ............................................. 38
11. Humane Endpoint Criteria ................................................. 43
12. Monitoring Animal Numbers on Protocols ................................. 43
13. Tracking Animals on Protocols .......................................... 45
14. Guidelines for Animal Transfers .......................................... 46

F. Mechanism for Receipt and Review of Concerns Involving Care and Use ............................................. 46

G. Euthanasia ........................................................................ 48

1. Methods of Euthanasia ...................................................... 48
   a. Physical Methods .......................................................... 48
   b. Non-physical / Pharmacological Methods ............................. 48
2. Hierarchy of Euthanasia Techniques ....................................... 48
   a. Disapproved Methods ............................................... 49
   b. Intra-cardiac Injections ............................................. 49
   c. Decapitation ............................................................... 49
   d. Cervical Dislocation ................................................ 49
   e. Exsanguination .......................................................... 50
   f. Carbon Dioxide Inhalation ........................................... 50
   g. Isoflurane Euthanasia / Drop Jar .................................. 51
   h. Isoflurane Euthanasia / Precision Vaporize ...................... 51
   i. Pithing ................................................................. 52
   j. Reducing Animal Anxiety During Euthanasia ..................... 52
   k. Weight Loss as an Endpoint ........................................... 52

H. Surgery ........................................................................... 53

1. Sterile / Aseptic Technique ................................................ 53
2. General Anesthesia .......................................................... 53
3. Regional / Local Anesthesia ............................................... 53
4. Anesthesia and Analgesia .................................................. 54
1. **Husbandry** ......................................................... 82
   1. **Animal Acclimation after Arrival** ......................... 82
J. Additional Considerations Pertaining to Protocols

1. Antibody Production ................................................................. 102
   a. Polyclonal Antibody Production ............................................. 103
      i. Immunization Technique ....................................................... 103
      ii. Use of Complete Freund’s Adjuvant and Incomplete Freund’s Adjuvant ......................................................... 103
      iii. Reducing Complete Freund’s Adjuvant Side Effects ................. 103
      iv. Complete Freund’s Adjuvant and Incomplete Freund’s Adjuvant Injections ......................................................... 103
      v. Complete Freund’s Adjuvant as a Health Hazard to Humans ...... 104
vi. Alternatives to Complete Freund’s Adjuvant and Incomplete Freund’s Adjuvant .............................................................. 104
vii. Choosing the Immunization Route .............................................. 104
viii. Spacing Immunizations ............................................................. 104
ix. Blood Collection ........................................................................ 105

b. Monoclonal Antibody Production .................................................. 105
i. Two Uses of Animals in Generating Monoclonal Antibodies .......... 105
ii. Non-Animal Alternatives ............................................................... 105
iii. Guidelines for Using Animals for Hybridoma Expansion ............. 106
iv. Guidelines for Using the Ascites Collection Technique ................. 106

K. Appendices. .................................................................................. 109
1. Protocol Flow Chart Time Lines ..................................................... 110
2. Amendment Flow Chart Time Lines ................................................. 111
A. What Is The Georgia State University IACUC?

The Institutional Animal Care & Use Committee (IACUC) is a standing committee at Georgia State University that is charged with overseeing the safety, respect, and dignity of animal subjects involved in scientific research in compliance with applicable federal regulations and guidelines. In addition, the impact to human health must be considered when interacting with animals. This process occurs via a cooperative effort between the IACUC, principal investigators, laboratory staff, Research Occupational Health and Safety, and the Department of Animal Resources. The Georgia State University IACUC uses the *Guide for the Care and Use of Laboratory Animals*, 8th Edition as the main guidance document. Many sections of this Policy and Procedure Manual have been taken directly from the *Guide*.

Membership of Georgia State University’s IACUC meets the compositional requirements and recommendations set forth in the Animal Welfare Act (AWA) Public Law 98-198)) and Public Health Service (PHS) policy, which states that the IACUC must consist of at least five members who are appointed by the institution’s chief executive officer (CEO). At Georgia State University the President of the University (CEO) has delegated appointment authority to the Vice President for Research and Economic Development. This individual is also the Institutional Official (IO) for Georgia State University. The appointed members must be qualified through experience and expertise to provide oversight for the institution’s animal programs, facilities, and procedures.

Committee membership includes the following:

- a Doctor of Veterinary Medicine either certified (e.g., by ACLAM, ECLAM, JCLAM, KCLAM) or with training and experience in laboratory animal science and medicine or in the use of the species at the institution
- at least one practicing scientist experienced in research involving animals
- at least one member from a nonscientific background, drawn from inside or outside the institution
- at least one public member to represent general community interests in the proper care and use of animals

The Attending Veterinarian is a permanent appointment to the IACUC. The other members shall serve three-year appointments. Membership vacancies shall be filled for compliance with the regulations and, to the extent possible, with similarly qualified individuals. All committee members are required to sign and abide by a Confidentiality Agreement yearly.

The Georgia State University IACUC can utilize consultants, when required, for specific protocol review. The consultant may offer opinions and advice, but may not vote on any application for use.

All IACUC minutes and reports are provided to the Institutional Official (IO).

1. IACUC Oversight

As a decision making body of Georgia State University, the IACUC ensures that all animals in experimental research or teaching are used appropriately and treated in accordance with the highest standards of humane care.

The IACUC represents society’s concerns regarding the welfare of animal subjects and is expected to be the conscience for the institute on animal welfare concerns.
The IACUC is responsible for keeping abreast of changes in animal use legislation and guidelines and recommending modifications to the institution's program to ensure that research and the animal use program fully comply with the letter and spirit of the law.

While the responsibility for scientific merit review normally lies outside the IACUC, the committee members must evaluate scientific elements of the protocol as they relate to the welfare and use of animals. All funded projects should have received appropriate, mission related and scientifically sound reviews (e.g. by the grant review process) apart from review by the IACUC. Humane treatment and scientific methodology, however, are closely related and often inseparable concepts. Therefore, the Committee may discuss and review science only as it relates specifically to animal use.

The IACUC expects that for all animal activities at Georgia State University, animal welfare is a primary focus. In addition, the impact to human health must be considered when interacting with animals. However, not all animal activities are regulated in the same manner.

**Full protocol:**
A full protocol is required for any teaching, testing or research performed on animals at Georgia State University. Larval forms of fish and amphibians have vertebrae and are covered by the PHS Policy and thus must have a full protocol. If live animals are used to produce the eggs, that activity must be covered under an IACUC protocol.

Any changes to the approved animal activities must be addressed by submitting a protocol amendment for review and approval before the new activity occurs. Full protocols are subject to the requirement for certification of participating personnel, semi-annual inspections, completion of annual renewals for AWA covered species, and de novo review after three years, among other requirements.

**Not all animal activities require a protocol. If the animal activity falls under one of the categories listed below, please contact the IACUC Office.** The IACUC Office will review the inquiry and determine whether the activity falls into one of the categories listed below, or whether the activity must be submitted as a full protocol. The PI is responsible for notifying the IACUC Office if the animal activity changes in a substantive way.

**Collaborations:**
- There are many circumstances that involve partnerships between collaborating institutions or relationships between institutional animal care programs. OLAW and APHIS agree that review of a research project or evaluation of a program or facility by more than one recognized IACUC is not a federal requirement. If both institutions have full PHS Assurances, they may exercise discretion in determining which IACUC reviews research protocols and under which institutional program the research will be performed. When collaborative research by a Georgia State University investigator is to be completed at another institution, a Collaborative protocol is required and a copy of that institution's approved protocol and approval letter is obtained and maintained at the IACUC Office.

**Wildlife:**
Activities where free-living wild vertebrates are observed and/or monitored undisturbed in their natural habitat.

Activities involving specimens that can be collected without handling or otherwise interfering with an animal or its environment such as scat, discarded feathers, hair or fur.

AWAR §2.31,d,1 states that field studies are exempt from IACUC review. PHS-funded field studies are not exempt. However, the IACUC must review field studies to determine whether the proposed activities involve an invasive procedure or harm or materially alter the behavior of the animals under study. Appropriate permits are required for all activities involving wildlife.

Eggs:

- Although avian and other egg-laying vertebrate species develop backbones prior to hatching, OLAW interprets the PHS Policy as applicable to their offspring only after hatching. The egg-laying adult animal is covered under an IACUC protocol.

AWAR does not regulate birds or cold-blood animals such as lizards, snakes or frogs. Therefore, there are no AWAR requirements for the IACUC to review the use of eggs from these species. OLAW has interpreted “live vertebrate animals” to apply to egg-laying vertebrate species only after hatching (ILAR News, 1991 33:68-70). Based on this interpretation, the same stage of development in fish is considered to be when the embryo has absorbed the yolk sac or begins to forage on its own (ILAR Journal, 2003 44:286-294).

No live animals:

- Research, teaching or testing activities involving carcasses, tissues, cells or fluids. Note that live animals may not be manipulated expressly for the purpose of obtaining the material. Although AWAR §1.1, §2.31 define regulated animals as “live or dead”, a “research facility” is defined as one using or intending to use only live animals and there is no reference to the use of dead animals. Therefore, the usual interpretation of AWAR §1.1 and §2.31 is that IACUC review is not required for activities involving dead animals. Similarly, only live animals are referenced in PHS Policy III,A. Therefore, PHS Policy does not require IACUC review and approval of the use of dead animals that are not specifically manipulated or euthanized for that activity. Also see: http://grants1.nih.gov/grants/olaw/references/laba97v26n3p21.htm.

Material can be obtained from:

- An animal that is euthanized as part of another approved IACUC protocol
- Another research institution
- Commercial sources, including scientific suppliers, tissue or blood banks, supermarkets, or abattoirs.*

* Does not include custom antibodies or other bio-products produced specifically for the investigator using live animals in any way
Samples used for diagnostic tests performed by private veterinarians or diagnostic laboratories
Salvaged animals (e.g., road kill, euthanized by private veterinarians or animal shelters).*
* Salvaged wildlife requires appropriate permits to be obtained by the PI.

Activities Exempt from an IACUC protocol
- Activities involving retrieval or use of animal-related data from records
- Any activity involving invertebrate species

Note:
Standard veterinary care, as performed by the DAR veterinarians and staff, does not constitute research, teaching or testing. Rather, these activities are part of the animal care program, which is reviewed by the IACUC during the semi-annual program review.

All animal activities are subject to IACUC guidelines, policies and procedures, in addition to all regulations imposed by any local, state or federal agency (e.g., wildlife permits). If IACUC approval of a study is required by a regulatory or funding agency a full protocol must be submitted.

The PI must ensure that all personnel are appropriately enrolled in the Medical Monitoring Program and ensure that they receive proper/adequate training appropriate to the scope and nature of the activities.

If the study activities change in any way, the IACUC must be immediately notified in writing of the changes.

Georgia State University is registered as an animal research facility with NIH and USDA. Georgia State University Animal Welfare Assurance (A3914-01) states that “This Assurance is applicable to all research, research training, experimentation, biological testing, and related activities, hereinafter referred to as activities, involving live vertebrate animals supported by the Public Health Service (PHS) and conducted at this Institution, or at another institution as a consequence of the sub-granting or subcontracting of a PHS conducted or -supported activity by this Institution.”

2. Principal Investigator Responsibilities
A PI must have the experience, professional qualifications, and access to the research facilities and resources necessary to ensure the proper care and use of vertebrate animals in research and/or teaching. For purposes of the submission of all research proposals involving vertebrate animals at Georgia State University, the IACUC recognizes only one individual as the PI. All other investigators on the protocol are considered co-investigators or key personnel.

These accountabilities apply to all persons who accept the responsibility as Principal Investigator to conduct research using vertebrate animals at Georgia State University or any of its affiliate institutions. This includes faculty, staff, and students at the institution.

The Principal Investigator must:
Comply with the Animal Welfare Act Regulations (AWRs [CFR 1985]), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy [PHS 1996]), U.S. Department of Agriculture, and other applicable federal, state, and local laws, regulations, and policies. The Guide for the Care and Use of Laboratory Animals (The Guide, Eight Edition NRC 2011) outlines all the PHS requirements followed by the IACUC.

**The Guide, Ethics and Animal Use:**

“The decision to use animals in research requires critical thought, judgment, and analysis. Using animals in research is a privilege granted by society to the research community with the expectation that such use will provide either significant new knowledge or lead to improvement in human and/or animal well-being (McCarthy 1999; Perry 2007). It is a trust that mandates responsible and humane care and use of these animals. The Guide endorses the responsibilities of investigators as stated in the U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.” (p. 4)

- Establish a culture of compliance; zero tolerance for non-compliance with established policies, standard operating procedures, and regulations
- Conduct the study in accordance with the written protocol approved by Georgia State University IACUC
- Abide by the Protocol’s Certification of Compliance statement
- Comply with all Georgia State University IACUC policies, procedures, decisions, conditions and requirements
- Ensure that the PI and all animal handlers on the research team read the applications and amendments after approval and be familiar with all animal procedures
- Maintain oversight of the research protocols and research staff
- Establish a 24-hour contact number so that the Division of Animal Resources (DAR) can contact lab personnel at any time to ask animal health related questions
- Ensure that all members of the research team (including the PI) are adequately trained, both initially and throughout the course of the study. This includes students and other trainees, visiting scientists, and volunteers who are directly involved with the project.
- Work closely with the Georgia State University veterinarians for animal care issues, especially if complications arise
  - Per the Guide (p.114): In the case of a pressing health problem, if the responsible person (e.g., investigator) is not available or if the investigator and veterinary staff cannot reach consensus on treatment, the veterinarian has the authority to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary.
- Maintain complete records and documentation appropriate to the type of research
• Participate in the Post Approval Monitoring (PAM) program as requested by the Office of Research Integrity
• Implement appropriate safety practices and procedures while working with hazardous agents or materials for lab staff and DAR staff.

The Principal Investigator must:
• Submit a new protocol and the associated grant, if applicable, for all research and/or teaching involving vertebrate animals to the IACUC for review by the first business day of the month. Research and/or teaching cannot begin until all requested changes or clarifications are described in the revised protocol, all paperwork/approvals from other Compliance Committees (i.e., Institutional Biosafety Committee) have been received by the IACUC Office, and all training has been completed by the PI and laboratory personnel. After all the requirements have been met, the IACUC Office will generate a letter of approval; the PI cannot begin the project until he/she receives written notification.
• Submit revised protocols and new applications to IACUC on time
• Submit an amendment request for all proposed protocol modifications before any changes are initiated. Animal related work associated with an amendment cannot proceed until the PI receives written notification from the IACUC that the proposed change(s) have been approved.
• Complete the appropriate paperwork for annual review of all covered animal approved protocols to receive continued protocol approval.
• Submit a new protocol for work that will continue beyond the three year (with annual review) approval period.

Ensure that research personnel adhere to procedures described within approved animal use applications, IACUC policies, and DAR policies
• Review new and revised IACUC policies with research staff, for example:
  o Proper CO₂ euthanasia
  o Amendments required for new procedures and personnel
  o Social housing for social species
  o Proper procedures in DAR facility
  o Proper transport of animal(s)
  o Carcass disposal
  o No sharing of animal facility access cards
  o Ensure proper documentation: The USDA and PHS policies require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. Dates of all observations, treatments, and procedures must be recorded. Dates and times (including AM/PM) of all time-sensitive observations or treatments (post-operative evaluations, pain medication) must be recorded. The extent of the records vary based on the nature of the procedure; however, at a minimum, records of the procedure must consist of: Animal ID, date of procedure, type of procedure, anesthetics/analgesics used (dose, route, time), anesthesia chart (vital signs – e.g. pulse rate, heart rate), drugs given (dose, time), general procedures (e.g.
intubation, beginning and end of surgery, etc.). Any deviations from the procedure as approved in the protocol due to emergency need must be documented, explained, and reported to the Institutional Animal Care and Use Committee. All records must be available for review at any time by IACUC and external regulatory officials.

**Protocol Compliance**
- Conduct and document appropriate monitoring of animals as deemed indicated in the approved protocol
- Report to the IACUC unanticipated adverse effects occurring in experimental animals

**Laboratory Personnel Who Use Live Vertebrate Animals**

The Investigator must ensure the adequacy of training and the level of experience of all animal handlers in the laboratory. The Investigator must ensure that all individuals in their laboratory who handle animals either have the necessary training or will receive the training necessary to conduct procedures according to federal regulations and IACUC policy. The training may be conducted by DAR technicians and veterinarians or other approved investigative staff. All animal handlers must be listed on the appropriate animal use protocol application. Completion of the on-line training does not add personnel to an animal use protocol application. This must be done when the animal use protocol application is initially submitted or later as a personnel amendment.

**Required Training, Enrollment in the Medical Monitoring Program, Animal Facility Orientation and Addition of Personnel Listed on the Animal Use Application**

Before research begins on an approved IACUC animal use protocol there are four steps to be completed by every person working with live vertebrate animals at Georgia State University: 1) complete the AALAS Learning Library IACUC online training; 2) attend an animal facility orientation meeting; 3) enroll in the Medical Monitoring Program; and 4) document training experience and/or receive hands-on training, if needed. The following outline briefly describes each of these steps and special considerations.

**I. AALAS Learning Library IACUC Online training**

Before handling animals, the individual must complete the appropriate courses, based on the species of animals used and the procedures that will be done, in the AALAS Learning Library IACUC Online training. Online training requirements are located via [http://ursa.research.GSU.edu/ursa/compliance/iacuc/requirements-for-working-with-animals/](http://ursa.research.GSU.edu/ursa/compliance/iacuc/requirements-for-working-with-animals/)

**II. Animal Facility Orientation**

These orientations are offered on a monthly basis. To schedule participation in an animal facility orientation meeting, please contact the Department of Animal Resources at 404-413-3560. Animal Facility orientation is required for all new animal users. Access will not be granted to the animal facility until the user has undergone the orientation process. The orientation is not required for established users of the animal facility. Rather, the targeted individuals are those new to the Georgia State University animal facilities.
III. Enrollment in the Medical Monitoring Program for Vertebrate Animal Exposure (MMPVAE)

The National Institutes of Health (NIH) requires that each university receiving federal support for research involving vertebrate animals develop and implement a Medical Monitoring Program for personnel with exposure to laboratory animals. Enrollment in the MMPVAE is online (http://ursa.research.GSU.edu/ursa/compliance/research-occupational-health-and-safety/). Follow the directions on the form for submission. Please note that enrollment in the program (completion of the MMPVAE Enrollment Form: http://mmpvae.gsu.edu) is mandatory. Re-enrollment in the Medical Monitoring Program must be done annually.

IV. Hands-on and Species Specific Animal Handling and Procedure Training

Arrangements for hands on training are made through the Department of Animal Resources at 404-413-3560. Examples of available hands on training (not an inclusive list):

1. **DAR Mouse Workshop** – includes various basic techniques such as handling, identification, blood withdrawal, and injections.
2. **DAR Rat Workshop** – includes various basic techniques such as handling, identification, blood withdrawal, and injections.
3. **DAR Syrian Hamster Workshop** – includes various basic techniques such as handling, identification, blood withdrawal, and injections.
4. **DAR Siberian Hamster Workshop** – includes various basic techniques such as handling, identification, blood withdrawal, and injections.
5. **DAR Cotton Rat Workshop** – includes various basic techniques such as handling, identification, blood withdrawal, and injections.
6. **DAR Introduction to Surgery Workshop** – includes surgical site preparation, aseptic technique, instrument handling, incision creation, suturing, and wound clip application and removal.
7. **DAR Ovariectomy Workshop** – includes hands-on training of an ovariectomy (removing the ovaries) from an anesthetized lab animal, including revisiting techniques from the Introduction to Surgery Workshop.
8. **DAR Castration Workshop** - includes hands-on training of a castration (removing the testicles) from an anesthetized lab animal, including revisiting techniques from the Introduction to Surgery Workshop.

B. Required Educational Program on Animal Care and Use

The completion and documentation of all training, including generic as well as species- and procedure-specific training, is required prior to approval of the animal protocol by the Institutional Animal Care and Use Committee. All researchers, staff and students who interact with animals in the performance of research or assisting in research must complete a required educational program before the IACUC may approve a protocol. Targeted individuals include the principal investigator (PI), co-investigators, instructors, staff, students and others working with animals in association with this protocol. The online training modules must be repeated at three-year intervals.

The targeted individuals must be identified on the Animal Use Protocol. The animal procedures each individual is to conduct must be delineated and their experience (credentials) and competency, relevant to these procedures, must be indicated. If such experience is lacking, the investigator must indicate how the individual will be trained in the conduct of these procedures.
This requirement includes the principal investigator, co-principal investigators, and other key personnel who are responsible for the design and/or conduct of the study. The requirement also applies to sub-contractors, consultants, individual fellowship applicants, study coordinators, and persons who conduct procedures or assist with animal care and use in research. Graduate and undergraduate student research assistants are required to complete the training program.

1. **Facility Access**

Facility access will be granted once an individual has completed the required training, enrolled in the Medical Monitoring Program and has attended a Facility Orientation session presented by the Department of Animal Resources.

Access will be granted by DAR by activating an individual's Panther Card which will be used to open the swipe card locks for the animal facility.

Still and video photography is not permitted in animal facilities without express permission from the IACUC, the PI and DAR.

2. **Online Training**

Georgia State University utilizes the AALAS (American Association for Laboratory Animal Science) Learning Library as the online source for training. All targeted individuals can access the AALAS Learning Library at the following website: [http://www.aalaslearninglibrary.org/](http://www.aalaslearninglibrary.org/). This online training resource is provided at no charge to Georgia State University animal users.

For the initial sign up, click on “Enroll Now” on the AALAS Learning Library home page. Select that you are enrolling as “Myself” with an access code. Use the access code "trainingGSU" (not case sensitive) to set up a personal Log-In ID and Password.

   a. **Required Modules**

Required training modules are dependent upon the species with which one works as well as the procedures which one will perform. The required species- and procedure-specific training can be found on the Georgia State University Animal Care and Use website [http://ursa.research.gsu.edu/ursa/compliance/iacuc/requirements-for-working-with-animals/](http://ursa.research.gsu.edu/ursa/compliance/iacuc/requirements-for-working-with-animals/) under the link entitled “Required Education.” [http://ursa.research.gsu.edu/repo/Required_Education.doc](http://ursa.research.gsu.edu/repo/Required_Education.doc).

   b. **Training Module Completion**

The IACUC is automatically notified of the individual’s successful completion of any module offered on the AALAS Learning Library website.

---

**Failure to complete and/or pass the training modules** could result in revocation of your protocol approval for research or other action(s) deemed appropriate by IACUC.

If you have any questions regarding this requirement, please contact the Georgia State University IACUC Office at 404-413-3508 or by e-mail at iacuc@GSU.edu

**Required Training Frequently Asked Questions**

The questions below are provided as a general guideline to aid in determining what training needs to be completed, who needs to be trained and how additional
students, staff, and researchers should be added to animal protocols. If you have questions please contact the Georgia State University IACUC Office at 404-413-3508 or by e-mail at iacuc@GSU.edu

Click on a question below to jump to the full answer:

1) Who needs to complete training?
Any individual that will have direct contact or interaction with animals. This includes project staff, student assistants, technicians, post-docs, and researchers observing procedures or research testing. OLAW (AWR 2.32) requires training for those caring for, treating or "using" animals in research activities. Any PI or co-PI that is listed as such on the protocol must also complete the training, even if they will not have direct animal contact.

2) I regularly send data to a researcher at another institution for analysis. The researcher does not see, interact, or handle the animals. Does he/she need to complete the training?
No. If an individual is only receiving data and will not directly work with or observe the animal procedure, they do not need to complete the training. However, this individual needs to be added to the protocol as a collaborator.

3) How do I add additional personnel to my protocol?
Once an individual completes training, the principal investigator completes a Personnel Amendment form. Once training is verified and

Examples:

i. Colleague uses computer models to simulate the learning phenomena obtained from the animals… (Click for full question & answer)

ii. Colleague who designs studies to run in my lab… (Click for full question & answer)

iii. Studies designed by my colleagues’ student… (Click for full question & answer)

iv. Data collected on animals to be compared to others data… (Click for full question & answer)

v. Software designed here and used elsewhere… (Click for full question & answer)
approved, the individual will be added to the protocol and an approval letter will be sent to the principal investigator. Currently the IACUC has a “paper” system (for all protocols submitted prior to September 1, 2015 and an electronic system for all protocols submitted on or after September 1, 2015. To add personnel to a paper protocol please use the Personnel Amendment Form found on the IACUC web page: http://www.GSU.edu/images/vp_research/GSU_IACUC_Personnel_Amendment_Form.doc. To submit a Personnel Amendment in the electronic system please go to https://gsu-iacuc.imedris.net.

4) Who can submit a Personnel Amendment Form for Animal Protocols?
Only the principal investigator of the protocol can add personnel to a protocol. The form must be signed by the principal investigator.

5) I want to use data from an experiment with students in a class to teach them how to analyze research data. The students will not work with or interact with the animals. The analysis is for a class project only and will not result in a publication. Do my students need to complete training and/or be added to my protocol?
No. Because the students will not be observing procedures or working or interacting with the animals they do not need to complete the training. In addition, because the data is being used for teaching (not for publication) the students do not need to be added to the principal investigator’s protocol.

6) I have a colleague, who uses computer models to simulate the learning phenomena obtained from the animals. Although she will never physically test the animals, the papers we publish will certainly have animal data and simulation data. Does she need to complete the training?
No. She does not need to complete the training.

7) I have an offsite collaborator that designs studies, such as a computerized task, and sends the tasks to Georgia State University for testing with the animals. This collaborator does not actually conduct the testing. Rather, he writes programs and receives data. Does he need to complete the training?
No. He does not need to complete the training.

8) My collaborator’s graduate students assist him with the research (to varying degrees, from data management to actual experimental design and analysis). The students would probably appear as co-authors on papers in which Georgia State University animal data are reported. Do these students need to complete the training?
No. The students do not need to complete the training.

9) As a principal investigator I collect animal data under my approved protocol at Georgia State University. This data will be compared and published with data collected on humans by a colleague. Does my colleague need to complete the training?
No. Your colleague does not need to complete the training.
I program software to be used in testing animals in my approved research protocol at Georgia State University. If I provide my software to other researchers to use, do they need to complete the training?

No. They do not need to complete the training.

C. Occupational Health and Safety Program Related to Animal Care and Use

The Research Health and Safety Program is tasked with overseeing and managing the Medical Monitoring Program for personnel with exposure to laboratory animals. The National Institutes of Health (NIH) requires that each university receiving federal support for research involving vertebrate animals develop and implement a Medical Monitoring Program for personnel with exposure to laboratory animals.

The purpose of the program is to prevent, monitor, and reduce diseases transmitted from animals to humans (zoonotic diseases) and mitigate adverse reactions from exposure to laboratory animals (e.g., allergies). In addition, educational programs have been established to educate personnel about zoonotic diseases, personal hygiene, and other related issues. Research Health and Safety also involves applying a combination of laboratory practices and procedures, laboratory facilities, and safety equipment when working with potentially hazardous materials to protect laboratory personnel, research products, the environment, and public health.

All animal users must enroll in the Research Occupational Health and Safety Program (ROHSP). To do so, it is necessary to complete the Medical Monitoring Program for Vertebrate Animal Exposure (MMPVAE) Enrollment Form as found on the ROHSP web page (http://ursa.research.GSU.edu/ursa/compliance/research-occupational-health-and-safety/). Follow the directions on the form for submission. Please note that enrollment in the program (completion of the MMPVAE Enrollment Form: http://mmpvae.gsu.edu) is mandatory. Re-enrollment is required annually.

D. Protocol Review and Approval

The IACUC shall oversee the use of all live vertebrate animals by Georgia State University, whether for research, instruction, production (breeding), or health surveillance purposes. Investigators using live vertebrate animals in such activities are required to submit an animal use protocol for IACUC review and approval before animals can be procured for such activities and facility access can be granted.

Principal Investigators must submit new protocols to the IACUC Office for initial processing and to the campus veterinarian for consultation by the first business day of the month to be eligible for review at the IACUC monthly meeting. In addition, the PI must submit the revised protocol (revisions recommended by the vet) back to the IACUC Office by the 10th business day in order for it to be eligible for review at the IACUC monthly meeting.

The IACUC will ensure that the proposed projects are in accordance with this Policy (PHS Policy IV.C.). Further, the IACUC will “…confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the Guide unless acceptable justification for a departure is presented” (USDA Animal Welfare Act 9 CFR, Subchapter A).

In accordance with PHS policy and USDA regulations, this institution provides all IACUC members the opportunity to review every animal care and use protocol and provide comments.
Although the IACUC has numerous responsibilities in terms of program oversight, the duty most identified with the IACUC is protocol review. The IACUC conducts a thorough and comprehensive review of all new proposals and amendments to existing protocols.

All continuing protocols also receive annual review, if an AWA covered species is utilized, to ensure that no significant deviations from established and approved procedures have occurred. The principal investigators are required to complete an annual review report as part of this process.

The animal use protocol is a detailed description of the proposed use of laboratory animals. The following topics are considered in the preparation of the protocol by the researcher and its review by the IACUC:

- rationale and purpose of the proposed use of animals
- a clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee
- availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation
- justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis)
- unnecessary duplication of experiments
- nonstandard housing and husbandry requirements
- impact of the proposed procedures on the animals’ well-being
- appropriate sedation, analgesia, and anesthesia (indices of pain or invasiveness might aid in the preparation and review of protocols)
- conduct of surgical procedures, including multiple operative procedures
- post-procedural care and observation (e.g., inclusion of post-treatment or postsurgical animal assessment forms)
- description and rationale for anticipated or selected endpoints
- criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated
- methods of euthanasia or disposition of animals, including planning for care of long-lived species after study completion. Methods of euthanasia are consistent with methods set forth by the American Veterinary Medical Association’s Panel on Euthanasia located at AVMA Panel on Euthanasia
- adequacy of training and experience of personnel in the procedures used, and roles and responsibilities of the personnel involved
- use of hazardous materials and provision of a safe working environment

IACUC protocols, new or three year de novo (renewals), are submitted via an electronic protocol management system (iMedRIS). A pre-review of the protocol is conducted by the IACUC Office staff for missing information or clarification of stated information. Veterinary review is then conducted on the submitted protocol. Prior to committee review, each IACUC member will be provided with written descriptions of activities (protocols) that involve the care and use of animals and any member of the IACUC may obtain, upon request, full committee review of those protocols.

No animal work may begin before the full committee has been given the opportunity to review the protocol and call for a full-committee review and before the protocol has been approved by (1) the majority of a quorum of the members or (2) the designated reviewer in the absence of a
call for full committee review. It is acknowledged that neither the PHS Policy nor the Animal Welfare Regulations recognize "provisional" or "interim" approval of any animal study proposal.

If full-committee review (FCR) is not requested, at least one member of the IACUC, designated by the chairperson and qualified to conduct the review, may be assigned to review those protocols and have the authority to approve, require modifications in (to secure approval) or request FCR of those protocols.

Other IACUC members may provide the designated reviewers with comments and/or suggestions for the reviewer’s consideration only. That is, concurrence to use the designated-member review (DMR) method may not be conditioned.

If multiple designated reviewers are used (two), their decisions must be unanimous; if not, the protocol will be referred for FCR.

If FCR is requested, approval of those protocols may be granted only after review at a convened meeting of a quorum of the IACUC and with the approval vote of a majority of the quorum present. Full Committee Review is conducted in person or in combination with a teleconference. Any use of telecommunications will be in accordance with NIH Notice NOT-OD-06-052 of March 24th, 2006, entitled Guidance on Use of Telecommunications for IACUC Meetings under the PHS Policy on Humane Care and Use of Laboratory Animals.

Generally, the FCR method will be used for most new protocols or three year renewals. However, should a situation warrant it, the protocol will be distributed to all IACUC members to allow all members the opportunity to call for FCR; records of polling of members to obtain concurrence to use the DMR method, or concurrence by silent assent after three working days, and approval of protocols via DMR are maintained and recorded in the minutes of the next convened IACUC meeting.

No IACUC member may participate in the IACUC review or approval of a protocol in which the member has a conflicting interest (e.g., is personally involved in the project) except to provide information requested by the IACUC; nor may a member who has a conflicting interest contribute to the constitution of a quorum. The IACUC may invite consultants to assist in reviewing complex issues. Consultants may not approve or withhold approval of an activity or vote with the IACUC unless they are also members of the IACUC.

Please note that all protocols classified as category E (using USDA promulgated pain/distress categorization) automatically go to the IACUC for Full Committee Review.

After the Full Committee has reviewed the protocol; the Chair (or Vice Chair) calls for the vote. A show of hands indicates “yes”, “no” or abstention.

- The possible outcomes of FCR are as follows:
  a. Approval
  b. Require Modifications to secure approval
  c. Approval Withheld

Review of Required Modifications Subsequent to FCR: When the IACUC requires modifications (to secure approval), of a protocol, such modifications are reviewed as follows:

a. FCR or Designated Member Review (DMR) following all the applicable procedures delineated in the PHS Policy.

OR
b. DMR if approved unanimously by all members at the meeting at which the required modifications are delineated AND if the entire current IACUC has previously approved, in advance and in writing, that the quorum of members present at a convened meeting may decide by unanimous vote to use DMR subsequent to FCR when modification is needed to secure approval. However, any member of the IACUC may, at any time, request to see the revised protocol and/or request FCR of the protocol.

If DMR is used, the approval date is the date the approval letter is generated for the final revised protocol after it has been approved by the designated reviewer(s). However, the approval date may not be more than 30 days after the DMR approved the final revised protocol.

Minor modifications of an administrative nature, i.e., typographical or grammatical errors, required signatures, etc. may be confirmed and conducted by IACUC administrative/support personnel.

Principal investigators should factor in adequate time (at least two months) for the protocol review and approval process.

In order to approve proposed protocols or proposed significant changes in ongoing protocols, the IACUC will conduct a review of those components related to the care and use of animals and determine that the proposed protocols are in accordance with the PHS Policy. In making this determination, the IACUC will confirm that the protocol will be conducted in accordance with the Animal Welfare Act insofar as it applies to the activity, and that the protocol is consistent with the Guide unless acceptable justification for a departure is presented. Further, the IACUC shall determine that the protocol conforms to the institution's PHS Assurance and meets the following requirements:

Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.

a. Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator.

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

c. The living conditions of animals will be appropriate for their species and contribute to their health and comfort. The housing, feeding, and nonmedical care of the animals will 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.

d. Medical care for animals will be available and provided as necessary by a qualified veterinarian.

e. Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures.

f. Methods of euthanasia used will be consistent with the current recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator.

Field Studies require an IAUC protocol. Investigations may involve the observation or use of non-domesticated vertebrate species under field conditions. Many field investigations require international, federal, state, and/or local permits, which may call for an evaluation of the scientific merit of the proposed study and a determination of the potential impact on the population or species to be studied.
Principal investigators conducting field research should be knowledgeable about relevant zoonotic diseases, associated safety issues, and any laws or regulations that apply.

In preparing the design of a field study, investigators are encouraged to consult with relevant professional societies and available guidelines. Veterinary input is needed for projects involving capture, individual identification, sedation, anesthesia, surgery, recovery, holding, transportation, release, or euthanasia. Issues associated with these activities are similar if not identical to those for species maintained and used in the laboratory. When species are removed from the wild, the protocol should include plans for either a return to their habitat or their final disposition, as appropriate.

1. Submission and Processing of Animal Use Applications
   All Protocol Submission and Amendment Forms can be found at: http://gsu-iacuc.imedris.net/

2. IACUC Meetings
   IACUC meetings are held on a monthly basis. See the online IACUC calendar for specific meeting dates and deadlines (http://ursa.research.gsu.edu/ursa/compliance/iacuc/iacuc-calendar/)

3. Duration of Protocol Approval
   For PHS purposes, the maximum interval between IACUC approvals for an ongoing activity is three years (PHS Policy at IV.C.5). There is no provision for IACUCs to grant administrative extensions of that time interval. Continuation of animal activities beyond the maximum approval period without such review would be a violation of PHS Policy and the terms and conditions of the NIH grant and violation of Georgia State University’s Assurance. Continuation of the project beyond three years requires submittal of a new protocol for review by the IACUC. Notifications of impending expirations are sent to principal investigators at around 3 months, 2 months, and 30 days in advance of the expiration date. Once a protocol has expired, a notification of expiration is sent to the principal investigator and copies of the notification are sent to the Animal Resources Office.

4. New Protocol Submission
   Principal investigators are required to complete and submit a new study to the IACUC for new activities involving the care and use of animals for research and teaching. In addition to the principal investigator, all other listed personnel working with animals on this protocol must have completed all appropriate animal care and use and species specific training. All such individuals must have enrolled in the Medical Monitoring Program. The instructions on how to complete the initial online training can be found in Section B of this Policy Manual. Instructions on how to enroll in the Medical Monitoring Program can be found on the Research Occupational Health and Safety web site http://ursa.research.gsu.edu/ursa/compliance/research-occupational-health-and-safety/

Consultation with a DAR veterinarian must occur on all protocols prior to review by the IACUC. This consultation can be in the form of a meeting, phone conversation, or electronic communication between the principal investigator (or the principal investigator’s representative) and the veterinarian. The veterinarian will review the protocol form and provide written comments so that revisions to the procedures, if suggested, can be made prior to formal IACUC review.
5. **Protocol Amendment Submission**

Principal investigators must complete and submit a Protocol Amendment Form in iMedRIS if the protocol was submitted after 9/1/2016 or via the paper form if the protocol was approved prior to 9/1/2016, if they propose any changes in ongoing active protocols. Any amendment(s) must be approved by the IACUC prior to initiating any changes to the protocol. If additional personnel are to be added, a Personnel Amendment Form must be submitted in iMedRIS if the protocol was submitted after 9/1/2016 or via the paper form if the protocol was approved prior to 9/1/2016. All personnel to be added must have completed all required training and be enrolled in the Medical Monitoring Program. The Personnel Amendment must be approved before new personnel may begin working on approved IACUC protocols. One personnel amendment form is required for each protocol, although multiple personnel may be added on each form.

As with new protocol submissions, consultation with a DAR veterinarian must occur on all protocol amendments prior to review by the IACUC. This consultation can be in the form of a meeting, phone conversation, or electronic communication between the principal investigator (or the principal investigator’s representative) and the veterinarian. After initial submission of the protocol amendment to the IACUC office, the veterinarian will review the protocol amendment and provide the PI with written comments so that revisions to the amendment, if suggested, can be made prior to formal IACUC review.

**Significant vs. Minor Changes**

ALL changes in ongoing active protocols must be submitted to the IACUC using the Protocol Amendment form in iMedRIS if the protocol was submitted after 9/1/2016 or via the paper form if the protocol was approved prior to 9/1/2016. The IACUC Chair and/or DAR veterinarian will make a determination if the changes are considered significant or minor using the following criteria which was compiled by the NIH Office of Laboratory Animal Welfare. Review and approval of significant changes is in accordance with the DMR process unless FCR is requested by an IACUC member.

Significant changes include changes that have, or have the potential to have, a negative impact on animal welfare. In addition, some activities that may not have a direct impact on animal welfare are also considered to be significant.

In support of the use of performance standards and professional judgment and to reduce regulatory burden, IACUC-reviewed and -approved policies (e.g., guidance documents, standard operating procedures, drug formularies) for the conduct of animal activities may be used for the administrative handling of some significant changes.

6. **Administrative Review and Approval**

Some significant changes to an IACUC protocol may be handled through the Veterinary Verification and Consultation (VVC) process according to IACUC-reviewed and -approved policies in consultation with a veterinarian authorized by the IACUC and the IACUC chair. This policy is in keeping with guidance provided by the Office of Laboratory Animal Welfare (NIH Guide Notice NOT-OD-14-126). The veterinarian is not conducting Designated Member Review (DMR), but is serving as a subject matter expert to verify that compliance with the IACUC-reviewed and -approved policy is appropriate for the animals in this circumstance. The consultation with the veterinarian and the IACUC Chair is documented. The veterinarian or the IACUC Chair has the authority to request IACUC review (via DMR or Full Committee Review) of the proposed changes for any
reason and must request such IACUC review for any changes which do not meet the parameters of this policy. IACUC members will not be provided with written descriptions of activities (amendments) that involve the care and use of animals for Administrative Review unless FCR or DMR is requested by the veterinarian or the IACUC Chair. Significant changes eligible for administrative review with VVC include*:

a. changes in anesthesia, analgesia, sedation, or experimental substances;
b. changes in euthanasia to any method approved in the AVMA Guidelines for the Euthanasia of Animals including those approved with conditions as long as the conditions are met; and
c. changes in the duration, frequency, type, or number of procedures performed on an animal.

*Please note that changes approved by the VVC process cannot be used to approve changes which result in greater pain or distress, a change in study objectives, an impact to personnel safety, or a change from a non-survival to a survival surgery.

Administrative Review by the veterinarian and the IACUC Chair may also include the additions of new strains of mice of the genus Mus and rats of the genus Rattus that are bred for use in research and significant changes in an increase in previously approved animal numbers, over 10% but less than 50%, in the case of a low number initially approved or no more than 100 total number of animals used of mice of the genus Mus and rats of the genus Rattus that are bred for use in research may be handled administratively. Such requests must address the rational for the original number of animals used, approved study objectives, the rational for the additional animals, and possible negative impacts on animal welfare. Requests and approval for these increases are documented.

Such policies

Changes of less than 10% in the approximate number of animals used of mice of the genus Mus and rats of the genus Rattus that are bred for use in research only may, at the IACUC’s discretion, be considered minor (not significant). These changes are, however, documented.

If additional personnel are to be added to an approved protocol, the addition of such personnel may also be handled administratively by the IACUC Chair. All personnel to be added must have completed all required training and be enrolled in the Medical Monitoring Program. Approval by the IACUC Chair must be documented prior to new personnel working on approved IACUC protocols.

Unforeseen Adverse Events

Fundamental to scientific inquiry is the investigation of novel experimental variables. Because of the potential for unexpected outcomes that may affect animal well-being when highly novel variables are introduced, more frequent monitoring of animals may be required. With their inherent potential for unanticipated phenotypes, Genetically Modified Animals (GMAs) are an example of models for which increased monitoring for unexpected outcomes could be implemented (Dennis 1999).

When unforeseen animal welfare concerns arise during the conduct of an IACUC approved animal procedure, the DAR Veterinarian must be consulted immediately. The
DAR Veterinarian will work with the Principal Investigator to find a solution that addresses the concern. The DAR Veterinarian is authorized to make an emergency protocol adjustment for humane reasons. Such a change must subsequently be approved by the IACUC via an amendment. Please note, however, that this does not imply that unapproved activities are condoned and should continue if interrupting them would not negatively affect the animals.

The DAR Veterinarian will send written confirmation detailing the emergency protocol adjustment to the Principal Investigator, the IACUC Office, and IACUC Chair within 3 business days of authorizing the protocol change. The Principal Investigator must submit a Georgia State University IACUC Protocol Amendment Form to the IACUC office within five (5) business days after receipt of the DAR Veterinarian’s written confirmation (please note: in the absence of submission of the Protocol Amendment within the 5 business days, the emergency protocol adjustment will expire).

7. Notification of Protocol Status
The PI receives notice, in writing, from the electronic protocol management system informing them of the status of the protocol following review. The PI will be notified whether the protocol or the amendment has been approved, requires modification or approval has been withheld.

If the protocol is approved
The electronic file is updated to note the date of approval and a letter of approval is sent via email notification to the PI. The approved protocol and/or amendment(s) are available to the Principal Investigator on the electronic protocol management system.

If the protocol requires modification
The investigator is informed of the modifications needed via the electronic protocol management system. The PI’s response is sent to the reviewers who have two (2) working days in which to determine if the investigator’s response is acceptable or if further detail is necessary.

If the response is acceptable to all designated reviewers, an approval letter is generated and sent to the PI.

If further detail is necessary, the investigator is notified and must submit a response. If the response is acceptable to all reviewers, an approval letter is generated and sent to the PI.

If approval is withheld
The investigator is informed of the withholding of approval and the notice will include the reasons why it was withheld.

If a principal investigator disagrees with the revisions required by the IACUC to obtain approval of a protocol, or with the disapproval of a protocol, the investigator may submit a written appeal to the IACUC stating the reasons for objecting to the required changes and/or proposing an alternative resolution. The principal investigator may also request a meeting with the IACUC to discuss the differences of opinion and resolve them. If no satisfactory resolution is reached, the principal investigator may submit a written appeal to the Institutional Official requesting assistance. The Institutional Official will attempt to
mediate a solution to the situation. Neither the Institutional Official nor any other administrative official, however, can override a decision made by the IACUC.

8. **Post-Approval Monitoring**

All ongoing activities are monitored continuously by the animal care and use staff and the IACUC Compliance Officer (post-approval monitoring). Post approval monitoring currently includes: program evaluations, reviews of protocols, reporting noncompliance, ensuring that individuals who work with animals are appropriately trained and qualified, and addressing concerns involving the care and use of animals at the institution. The veterinarians along with the animal care and technical staff, add another important level of program supervision including daily observation of animals by trained animal care personnel and communication to the veterinary staff for follow-up, facility monitoring by facility maintenance personnel, post-operative care by trained personnel, evaluation of outcomes of animal procedures by investigators and staff, hands-on training in animal procedures, and appropriate reporting of incidents involving occupational health and safety.

In addition, post-approval monitoring of research studies can be conducted to identify possible weaknesses and elicit process improvements. This strategy also serves to increase investigator awareness of regulatory requirements and improve the ethical conduct of research. This monitoring may be protocol oriented or investigator-oriented.

a. A protocol is randomly selected for observation to maintain consistency and evaluate procedures that have been submitted and approved as part of the IACUC process.

b. An Investigator is monitored based on known or suspected information regarding how procedures are conducted. Results can subsequently lead to monitoring of all active protocols approved for that investigator.

Investigators and laboratory staff will work with the IACUC Office to observe and confirm monitoring procedures with the approved protocol. The IACUC Office will work with the investigator and laboratory staff to observe research activity. Afterward accurate reports are prepared, recommendations for maintaining compliance is provided, and PIs are informed of training opportunities.

A checklist will be used for the routine post approval monitoring. During each post approval monitoring session, the IACUC Officer will compare procedures conducted in the laboratory with those listed in the approved protocol and any approved amendments. Documented discrepancies or disparities between the procedures performed in the lab and those listed in the protocol will be brought to the attention of the Principal Investigator in person and in writing.

Some of the discrepancies or disparities may include:

- Personnel performing procedures who are not listed in the approved protocol.
- Procedures performed in the labs that are not listed in the approved protocol.
- Anesthetics, analgesics, tranquilizers, antibiotics or other medications used in the lab that are not listed in the protocol, or different from those listed in the protocol, or not used in accordance with the protocol.
- Procedures listed in the protocol not being performed as approved in the protocol.
- Survival surgery that is not performed aseptically.
- Euthanasia procedures that differ from those listed in the protocol and/or a method for ensuring euthanasia that are not employed.

Other issues of concern may include:
- Lab personnel who appear to lack the necessary training to appropriately perform procedures listed in the protocol.
- Supporting documentation for animal care, post-op care or other study procedures that is incomplete or unavailable.
- Conditions not safe for humans and/or animals.
- The usage of outdated materials (drugs, suture, etc.).
- The use of un-calibrated equipment.
- Animal misuse, mistreatment or neglect (welfare issues), or discrepancies which result in animal welfare concerns. Deliberate animal misuse, mistreatment, or neglect, or those which involve willful disregard for appropriate animal care will be immediately reported to the IACUC, the Director of Animal Resources, and the Associate Vice President for Research Integrity. The report will be investigated by the IACUC following the IACUC procedures for handling non-compliance.
- Food, beverages, tobacco products and/or children in the research laboratory.

The IACUC Officer shall discuss monitoring results with the Principal Investigator and other lab personnel before leaving the laboratory as part of the exit interview. If the Principal Investigator is unavailable, the IACUC Officer will arrange for a time suitable with the Principal Investigator to return to discuss results. Issues that pose an immediate threat to animal welfare shall be referred to the IACUC Chair and a DAR veterinarian for immediate resolution.

The IACUC Officer shall send a written draft report of the monitoring results to the Principal Investigator. In all instances, investigators will have an opportunity to respond to the draft report before the final report to the IACUC is prepared. The IACUC Officer shall send a final written report of the monitoring results to the Principal Investigator, IACUC Chair and a DAR veterinarian. A copy of the report will be made available to the IACUC at their next, regularly scheduled meeting and the minutes will reflect the discussion of the results of the post approval monitoring.

The IACUC Officer will follow up on any issues that require protocol modifications, orientation of new personnel, or training. The IACUC Officer will support the laboratory corrective action by facilitating access to the required training and/or providing guidance for the revision of the protocol to bring it into current compliance. On occasion, additional monitoring sessions may be part of the follow-up to assist with proper corrective actions. Investigators who disagree with monitoring results and/or recommendations may appeal directly to the IACUC. A copy of the final compliance monitoring report shall be kept in the electronic file.

The IACUC Office ensures congruency between approved protocol applications and grant applications. All discrepancies or disparities are reported to the PI and, if necessary, are investigated by a subcommittee of the IACUC and may result in sanctions by the IACUC, which could include suspension of research activity.
9. Three Year Review of Protocols

Protocols are approved for a maximum of three years by the IACUC based on Public Health Service (PHS) Policy guidelines. Protocols with species covered under the AWA are subject to annual review through the IACUC Protocol Continuation or Cancellation Form (e.g. annual review form). Review and approval of this Form is in accordance with the Designated Member Review process unless Full Committee Review is requested by an IACUC member.

Expired Protocols with Animals Still Present

Outlined below is the procedure of what to do if a protocol approaches the expiration date with animals still active on the protocol.

1. Approximately four months prior to the protocol expiration date an email will be sent to the Principal Investigator concerning the upcoming protocol expiration. Consequences of protocol expiration are outlined in the email, including the consequences of having animals in the facility at the time of expiration.

2. Approximately two months prior to the protocol expiration date the protocol expiration The same email will be sent if no action has been taken to either renew the protocol or close the protocol at expiration.

3. Approximately one month prior to the protocol expiration date, the PI will receive a final email from the IACUC office concerning animals in the animal facility on expiring protocols.

4. For protocols with animals housed on University property - one week prior to the protocol’s expiration date if a new protocol has been submitted, but it does not appear that it will be approved by the expiration date, the IACUC Office will do the following:
   a. Notify the PI that:
      i. The animals will be transferred to a holding protocol at the PI’s expense prior to protocol expiration.
      ii. All husbandry activities will be performed by University Animal Care staff at PI expense, regardless of where the animals are housed. The exceptions are animals, such as aquatics, reptiles and some amphibians, that the PI normally takes care of (have no per diems associated with them) will still be taken care of by the PI, but no research may be conducted until the new protocol has been approved.
      iii. While on the holding protocol, no breeding, handling or other work with the animals may be performed unless.
   b. If the PI is currently breeding animals on the expired protocol, Division of Animal resources will separate all breeding pairs, at the PIs expense. Exceptions, such as preserving critical genetically modified lines, may be made, if it has been approved by the IACUC.
   c. The investigator and all staff listed on the expired protocol exclusively (not listed on any other active protocol) are denied access to the University Animal Care animal facilities as of the protocol expiration date.
   d. For those cases where a renewal protocol has not submitted prior to the expiration date, any animals left on the expired protocol will be euthanized. If the PI notifies the IACUC of the pending submission of the renewal, the
animals will not be held longer than 30 days. At the 30 day mark and no new protocol has been submitted the animals will be euthanized.

Upon approval of the new protocol, the animals are transferred from the holding protocol to the new protocol. Research activities may commence at this time

10. Suspension of Research Activity
The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present.

If the IACUC suspends an activity involving animals, or any other institutional intervention results in the temporary or permanent suspension of an activity due to noncompliance with the approved protocol, the Policy, Animal Welfare Act, the Guide, or the institution's Assurance, the Institutional Official, in consultation with the IACUC, shall review the reasons for suspension, take appropriate corrective action, and report that action with a full explanation to OLAW, USDA (if applicable) and AAALAC. IACUC may suspend an activity that it previously approved if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, the Guide, the institution's Assurance, or IV.C.1.a.-g. of the PHS Policy.
11. Protocol Flow Chart

IACUC Protocol Submission Process

1. PI Submits Protocol
2. Changes Required?
3. Vet 1
4. Changes Required?
5. Vet 2
6. Chair Assigns Reviewers
7. Reviewer 1 & 2
8. Discussed at IACUC meeting

Changes Required? (1st round of reviews)

Changes Required? (After 1st round of reviews)

Assigned to original reviewers if submitting corrections after review

Red Numbers
Associated Training Document
1. Create and Submit an IACUC Application
2. Pre-Review Screening
3. Assign Submission to a Vet
4. Complete Vet Consult Form
5. Process Vet Consult Form
6. How to Assign Reviewers
7. Complete a Reviewer Form
12. Amendment Flow Chart

Submit Amendment

Pre-Review

Send back for Corrections

Can Administrative Review be conducted?

Chair & Vet Conduct Review

Send back for Corrections

Has Vet Consult Been Completed?

Vet Consult is Conducted

Send back for Corrections

Has Vet already assigned reviewers?

Chair & Vet Conduct Review

Send back for Corrections

Previously assigned reviewers review corrected submission

No

Yes

No

Yes

Previously assigned reviewers review corrected submission

No

Chair assigns reviewers

Reviewers conduct review of amendment

Call for full board review

Yes

Assigned to agenda for full board discussion/review

No action required of board

Send back for Corrections

Approval processed

Notification sent to each IACUC member to review Amendment to call for full board review

Yes

Call for full board review

Yes

Assign to agenda for full board discussion/review

No action required of board

Send back for Corrections

No

Yes

Make Corrections

Yes

Make Corrections

No

Make Corrections

No

Make Corrections

Yes
E. Completing the IACUC Protocol Form

All new protocol submissions are made in the electronic protocol management system. To log onto this system go to https://gsu-iacuc.imedris.net. After logging into the system, click on the “Help” button for detailed descriptions on how to complete all available forms.

1. Regulatory Criteria

- **Activities**
  Must be in accord with USDA Regulations/PHS Policy and the Guide to the Care and Use of Laboratory Animals, 8th Edition.

- **Pain/Distress**
  Must avoid/minimize discomfort, distress, and/or pain. If pain/distress is caused, appropriate sedation, analgesia or anesthesia will be used. A veterinarian must be involved in planning. Use of paralytics without anesthesia is prohibited. Animals upon which teaching, experiments, research, surgery, or tests will be conducted involving accompanying pain or distress or leading to illness to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests must be approved by the IACUC. Animals with chronic/severe un-relievable pain will be euthanized.

- **Surgery**
  Must meet requirements for sterile surgery and pre/postoperative care. Cannot use one animal for more than one major operative procedures from which it will recover, without meeting specified conditions and approval by the IACUC.

- **Euthanasia**
  Euthanasia method must be consistent with the current American Veterinary Medical Association (AVMA) recommendations for that species.

- **Housing/Health**
  Living conditions for animals must be consistent with standards of housing, feeding and care per directives in PHS Policy, The Animal Welfare Act, the Guides to the Care and Use of Laboratory Animals, directed by the veterinarian or scientist with appropriate expertise and approved by the IACUC.

- **Alternatives**
  Federal Regulations (The Public Health Service Policy and the Animal Welfare Act) and University Policy require assurance that each project does not unnecessarily duplicate research projects/courses performed at this or other institutions, and that the use of alternatives to live animal models and alternative procedures that may cause more than momentary or slight pain/distress to animals have been considered. The 3Rs (Refinement, Reduction and Replacement)
must all be addressed.

- **Rationale and Methods**
  Must provide written narrative of methods/sources, rationale for using animals, and the reasons for using the requested species and the number of animals.

- **Duplication**
  Must provide assurance that activities do not unnecessarily duplicate previous efforts.

- **Qualifications**
  Personnel must be appropriately trained and competent.

- **Deviations from Requirements**
  Must be justified for scientific reasons, in writing.

2. **Why the Use of Animals in Research is Important**

This is a very important issue because you are asking for the privilege of using animals for procedures that rarely will benefit them individually, and almost always results in their death. In general, there must be a compelling potential for benefit to human or animal health to warrant the use of animals. Points to consider:

- If you are studying a human or animal disease or health concern, it is helpful to carefully explain the disease, what causes it, what therapy is currently used to treat it, and how the proposed animal experiments might better prevent human or animal pain and suffering. Explaining what you are doing (objective) and why you are doing it (rationale and significance) are all necessary.

- Because there are non-scientists on the IACUC, your response should be written so that members of the general public (including the lay members on the IACUC) would readily understand why it is important to use animals for your work.

- Make sure you explain medical terms and define abbreviations the first time they are used.

3. **Describing the Animal Studies**

In the description of the experimental design or activities involving animals, keep in mind that the IACUC needs to understand the proposed use of animals. It is important that one is able to ascertain what procedure or set of procedures is conducted on each group of animals, including the time frames and intervals between procedures. Description of the procedures in the order they will be performed is also important. To perform an appropriate review of your proposed animal work, IACUC members must understand what combination of procedures will be performed on an individual animal. Details of procedures such as surgery and euthanasia are required. Keep these points in mind:

- For more complex experiments, it is very helpful to provide a flow chart to make the experimental design clear.

- The description of the animal procedures should stand by itself. The IACUC should not have to read another document such as a grant application to understand what you propose.

- Define all abbreviations the first time they are used to facilitate comprehension.

Do not use technical language that only specialists in your field would understand. Not only is it difficult for trained professionals to navigate through technical jargon outside their fields, there are non-scientists and lay members serving on the IACUC.
4. **Species Selection**

The central theme evaluated by the IACUC is this - assuming that animals are indeed necessary, the least sentient ("aware") species capable of providing the needed data should be used. The hierarchy of sentient species can be a subject of disagreement, but generally is as follows:

- Apes (chimps, orangutans, gorillas)
- Monkeys (baboons, rhesus monkeys, marmosets, tamarins)
- Larger animals commonly kept as pets such as dogs and cats
- Larger animals such as pigs and goats commonly used as farm animals
- Rabbits
- Rodents (guinea pigs, hamsters, rats, mice)
- Non-mammalian vertebrates (poultry, amphibians, reptiles, fish)
- Invertebrates (crustaceans, slugs)
- Smaller life forms (insects, arachnids, worms)
- Single cell organisms (yeast, bacteria, etc.)

Only one species of animal may be listed on a protocol. Any work with multiple species requires multiple protocols or IACUC approval for any exceptions.

5. **Species Justification**

Justifications for using a particular species may include:

- The previous work in the biomedical literature validates the use of this species as an animal model for this disease or biological process.
- This is the lowest sentient species that provides appropriate size, tissue or anatomy of the proposed work.
- The existence of a large body of previous laboratory data that would have to be repeated if another species was used instead.
- Characteristics of the species that render it uniquely suited to the proposed research.
- Size, availability, and cost. Please note that cost savings alone is not an adequate justification for using a particular species. The justification should be based on sound scientific reasoning.
- Availability of reagents or research tools necessary for this research are unique to this species.

6. **Animal Numbers Justification**

You are asked to request a certain number of animals that will be used during the three year life of the protocol, and justify why you need that number. The IACUC realizes that it can be difficult to provide such information in advance.

Some important points:

- According to the Guide, a **statistical analysis** should be used to justify animal numbers. Commonly power analyses is the most appropriate tool for justifying group sizes, but consult a biostatistician for the best tools for your particular studies. You might even discover that you need to request more animals per group than you thought would be necessary.
- It is acceptable to request animals that will be used to perfect surgical or other techniques prior to initiating planned experiments. This is preferable to beginning a large experiment that will experience technical problems that might cause pain or distress to the animals.
- Studies on cadavers from other approved protocols in advance of any procedure on a live animal are strongly encouraged. By doing this,
techniques can be perfected as much as possible before any live animals are used.

- It is also acceptable to ask for animals that will be used in pilot experiments in addition to animals requested for more robust experiments. Pilot experiments can be used to perfect technique, demonstrate feasibility, or provide a justification for proceeding with larger, more tightly controlled experiments.
- For complex multiple procedure protocols, a table showing under what procedure animals will be used is often useful for the IACUC to understand the justification for the number of animals requested.

7. **The “Three R’s” - Replacement, Reduction, Refinement**

The concept of alternatives to animal use was first introduced in 1959 by the British scientists Russell and Burch (In: The Principles of Humane Experimental Technique, Methuen, and London). A responsibility of the IACUC (mandated by federal policy and regulations) is to ensure that animal users make appropriate efforts to consider and/or fulfill the three R’s.

**Replacement**
Substitute non-animal techniques for animal usage. Examples include:

- cell culture or tissue culture systems
- computer simulations
- *in vitro* assays such as immunologic bench assays to replace animal bioassays.

It is not very common for any of the above alternate systems to adequately replace animals in experiments. It is possible, however, and consideration should always be given to non-animal systems.

**Reduction**
Decrease the number of animals used for a particular activity or project whenever appropriate. Examples include:

- Limiting group sizes to the minimum needed to obtain statistically significant data.
- Performing multiple experiments simultaneously so that the same control group can be used for all the experiments.
- Sharing tissues with other investigators so that additional animals are not needed.
- Designing experiments so that animals serve as their own controls.
- Using newer instrumentation that improves precision and reduces the number of animals needed per data point.

Reduction is usually more feasible than replacement. When considering how to reduce animal use, however, you must find a balance between causing more pain or distress on fewer animals and causing less pain or distress in more animals. For instance, if an investigator proposes to double the number of invasive surgical procedures on animals so that fewer animals are used, the increased pain and distress experienced by the remaining animals may not be justified by a simple reduction in animal use. This is a difficult area, and you should seek advice from a DAR veterinarian and the IACUC as needed.
Refinement
Modify a technique or activity so as to reduce the pain and/or distress experienced by animals. Examples include:

- New anesthetics that allow rapid induction and reduced recovery times.
- New analgesics that provide more extended pain relief postoperatively with less frequent administrations.
- New bleeding and injection techniques that cause less tissue damage or distress.
- Improved surgical techniques that minimize trauma and the length of anesthesia.

Check literature and with your veterinarian concerning improved techniques that have evolved that reduce pain or distress on the animals.

U.S. animal welfare regulations define a painful procedure as one that “would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures.”

8. Search for Animal Alternatives
In accordance with the Health Research Extension Act of 1985, scientists performing painful and stressful experiments on animals must document if there are alternative methods to the painful procedure and report this information to the IACUC when they submit their animal use protocol form for approval. It is then the responsibility of the IACUC to determine if the alternative methods should be used. To assist IACUC’s and investigators in complying with this portion of the law, Congress established the Animal Welfare Information Center (AWIC) at the National Agricultural Library (10301 Baltimore Avenue, Beltsville, MD USA 20705-2351, Tel: 301 504-6212, Fax: 301 504-7125, email: awic@nal.usda.gov, http://www.nal.usda.gov/awic).

The regulations require, as a minimum, that an investigator perform a search of the literature in an attempt to identify alternatives to painful procedures. A multi-database approach is usually necessary, as an alternative procedure or method may originate from outside the specific discipline being studied.

Directory/websites for alternatives (examples)

OLAW
http://grants.nih.gov/grants/olaw/olaw.htm
Office of Laboratory Animal Welfare
National Institutes of Health (NIH)
RKL1, Suite 1050, MSC 7982
6705 Rockledge Drive
Bethesda, MD 20892-7982
Phone: (301) 498-7163
Fax: (301) 402-2803

Altweb
http://altweb.jhsph.edu
Altweb is a site for news, information, discussion, and resources from the field of alternatives to animal testing. This site is a collaborative effort funded by the alternatives Research & Development Foundation, the Doerenkamp-Zbinden Foundation, the Humane Society of the United States, the Office for Protection from Research Risks at the National Institutes of Health, and the Procter & Gamble Company. It is being developed by the Center for Alternatives to Animal Testing at Johns Hopkins University in collaboration with the Altweb Project Team, to serve academic, industrial and government scientists, educators, the media, and the general public.

Altweb is intended to foster the development of scientifically acceptable in vitro and other alternatives to animal testing. Alternatives are defined as methods that reduce animal use, replace whole animal tests, or refine existing tests by minimizing animal distress.

Animal Welfare Information Center
http://www.nal.usda.gov/awic/

United States Department of Agriculture (USDA)

This site provides access to:
- Full-text versions of all pertinent Federal laws, regulations, guidelines and policies, and links to international laws,
- AWIC newsletters,
- AWIC publications,
- Links to databases, information on alternatives, farm animals, and organizations,
- Links to the National Agricultural Library, Animal and Plant Health Inspection Service, Office for Protection from Research Risks, and NetVet.

Institute of Laboratory Animal Resources (ILAR) Journal
http://dels.nas.edu/ilar_n/ilarhome/index.shtml

ILAR Journal is the quarterly, peer-reviewed publication of the Institute for Laboratory Animal Research, which is a unit of the National Research Council, National Academy of Sciences. ILAR Journal provides thoughtful and timely information for all those who use, care for, and oversee the use of laboratory animals. Provides access to online version of the journal and many back issues; a searchable index is available.

Databases
Major databases include:
- AGRICOLA 1970 to the present
- CAB-INTERNATIONAL DATABASES 1972 to the present
- MEDLINE 1984 to the present
- CSA LIFE SCIENCES 1985 to the present

Core databases include:
- AT ALTERNATIVES circa 1920s to the present
- BIOLOGICAL VALUES
- BIOMEDICAL DISSERTAIONS
- BOOKS
- CABLINE
- CURRENT CITATION 1995 to the present
- DRUG DOSAGES
9. Unnecessary Duplication
The USDA Animal Welfare Act Regulations state that IACUCs must evaluate a written assurance that the proposed animal studies do not unnecessarily duplicate previous studies. You are asked to document that your proposed work is not unnecessarily duplicative on the IACUC forms.

The form of the written documentation is not specified by the Animal Welfare Act, but typically the same types of documentation used for the alternatives mandate do double duty here. **Experience has shown that database searches are effective ways to document that work proposed is not unnecessarily duplicative.**

Note that the critical concept is that unnecessary duplication is not allowed. Acceptance of new ideas in science is often dependent upon the ability of other scientists to duplicate published reports. The IACUC can allow duplication of previous work if you can convince them that it is important scientifically to do so.

10. USDA Pain/Distress Categories
The Georgia State University IACUC assigns all protocols to a USDA promulgated pain/distress categories as defined below. Please note that all category E protocols require review by a convened meeting of the full committee.

A simple yet useful **definition of a painful or distressful procedure on an animal** is this:

*A procedure that would cause pain or distress in a human.*

It is important to understand that if multiple procedures will be performed on an animal, the animal is placed in the category appropriate for the most painful/distressful procedure. One animal cannot be placed in multiple categories.

Minimizing animal pain, wherever possible, is important both ethically and legally. The National Academies have developed a free online resource to help those who care for and use laboratory animals, farm animals, and pets to prevent, recognize, and alleviate pain in different types of animals, from non-human primates to fish. Visit this online resource at: [http://nas-sites.org/animal-pain/](http://nas-sites.org/animal-pain/). The required online training also addresses pain recognition and minimizing pain and distress.

**Category “B”**

**Category B animals** are those that are being bred, conditioned, or held for use in research, teaching, or testing but not yet used for such purposes. These animals have not been used for any research procedure, however minor. **Category B** is the correct category for breeders and other animals that are not undergoing any experimental procedures. This category is also used for animals held under proper captive conditions or wild animals that are being observed.
**Category “C”**

**Category C animals** are those which teaching, research, experiments, or tests will be conducted involving no pain, distress, or use of pain-relieving drugs. Routine procedures performed correctly by trained personnel such as the administration of electrolytes/fluids, administration of oral medication, blood collection from a common peripheral vein per standard veterinary practice or catheterization of same, standard radiography, parenteral injections of non-irritating substances.

Other examples of category C procedures include:

- Euthanasia performed in accordance with the recommendations of the most recent AVMA Panel on Euthanasia;
- Utilizing procedures that produce rapid unconsciousness and subsequent humane death;
- Animals that are euthanized before tissue collection or other manipulations are also commonly placed in this category, if no other procedures are performed that would place them in a higher pain/distress category.
- Manual restraint, that is no longer than would be required for a simple exam; a short period of chair restraint for an adapted nonhuman primate would also fall into this category.

**Category “D”**

**Category D animals** upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress or leading to illness to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used.

Examples of category D procedures are:

- Surgical procedures conducted by trained personnel in accordance with standard veterinary practice such as biopsies, gonadectomy, exposure of blood vessels, chronic catheter implantation, laparotomy or laparoscopy;
- Administration of drugs, chemicals, toxins, or organisms that would be expected to produce pain or distress but which will be alleviated by analgesics.
- Blood collection by more invasive routes such as intra-cardiac or periorbital collection from species without a true orbital sinus such as rats and guinea pigs.
- Terminal exsanguination (euthanasia by removal of blood) under anesthesia. Painful, potentially painful, or distressful non-surgical procedures: e.g. bone marrow taps, injections into particularly sensitive areas such as foot pads, and cardiac punctures

**Category “E”**

**Category E animals** upon which teaching, experiments, research, surgery, or tests will be conducted involving accompanying pain or distress or leading to illness to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests can only be allowed if it is scientifically justified in writing and approved by the IACUC.

Examples of category E procedures include:
- Procedures producing pain or distress unrelieved by analgesics such as toxicity studies, microbial virulence testing, radiation sickness, and research on stress, shock, or pain,
- Surgical procedures and postsurgical sequella from the invasion of a body cavity, orthopedic procedures, dentistry or other hard or soft tissue damage that produces unrelieved pain or distress.
- Procedures such as negative conditioning using electric shock that would cause pain in humans and chairing of nonhuman primates who are not conditioned to the procedure for the time period used.

Category E studies are given increased scrutiny by the IACUC because it must be satisfied that less painful or stressful alternatives are not available, or that less painful/stressful endpoints cannot reasonably be used. By law, the institution must **annually report all category E procedures** to the USDA on USDA covered species and include a scientific justification supporting the IACUC’s decision to approve them. Often, the justification given by the investigator on the protocol form submitted to the IACUC is used for the annual report.

It is important for all information on category E procedures to be complete and accurate. Once this information is submitted to the USDA, it is available to the public.

11. **Humane Endpoint Criteria**

The experimental endpoint of a study occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress, including death. The humane endpoint should be relevant and reliable (Hendriksen and Steen 2000; Olfert and Godson 2000; Sass 2000; Stokes 2002). For many invasive experiments, the experimental and humane endpoints are closely linked (Wallace 2000) and should be carefully considered during IACUC protocol review. While all studies should employ endpoints that are humane, studies that commonly require special consideration include those that involve tumor models, infectious diseases, vaccine challenge, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, organ or system failure, and models of cardiovascular shock.

The PI, who has precise knowledge of both the objectives of the study and the proposed model, should identify, explain, and include in the animal use protocol a study endpoint that is both humane and scientifically sound. The identification of humane endpoints is often challenging, however, because multiple factors must be weighed, including the model, species (and sometimes strain or stock), animal health status, study objectives, institutional policy, regulatory requirements, and occasionally conflicting scientific literature. Determination of humane endpoints should involve the PI, the veterinarian, and the IACUC, and should be defined when possible before the start of the study (Olfert and Godson 2000; Stokes 2000).

Information that is critical to the IACUC’s assessment of appropriate endpoint consideration in a protocol includes precise definition of the humane endpoint (including assessment criteria), the frequency of animal observation, training of personnel responsible for assessment and recognition of the humane endpoint, and the response
required upon reaching the humane endpoint. Federal regulations require that IACUCs
determine that discomfort to animals will be limited to that which is unavoidable for the
conduct of scientifically valuable research, and that unrelieved pain and distress will only
continue for the duration necessary to accomplish the scientific objectives.

Humane endpoints refer to one or more predetermined physiological or behavioral signs
that define the point at which an experimental animal’s pain and/or distress is
terminated, minimized, or reduced by taking actions such as euthanizing the animal or
terminating a painful procedure. Humane endpoints function as an alternative to
experimental endpoints and provide investigators with an effective way to refine their
research. The establishment of humane endpoints prior to the start of a study allows the
investigator to prevent unnecessary animal pain and distress while ensuring accurate
and timely data collection. Humane endpoints should be clearly defined for each protocol
submitted to the IACUC for review.

The following default humane endpoints, adopted by the IACUC, will be applied only if
investigators do not delineate and adequately justify alternative endpoints. Prior to
submitting protocols to the IACUC, investigators are encouraged to develop more
refined endpoints that avoid or minimize discomfort, distress and pain to the animals and
that are compatible with experimental objectives.

Default humane endpoints for the following laboratory animals including rodents,
nonhuman primates, birds, frogs, and lizards.

**Rodents**

- Loss of 20% of body weight from baseline weight when assigned to the protocol.
  If protocol is utilizing a young growing animal, a growth nomogram must be used
to adjust the 20% weight deviation from a basal weight growing animals.
- Surgical complications unresponsive to immediate intervention; i.e. bleeding,
  vascular graft/circulation failure, infection, and wound dehiscence.
- Poor body condition score, 2 out of 5, (reference Manual Ullman-Culler MH Lab
  Animal Sci. 49(3):319-23, 1999) will either be selected for euthanasia or the
  condition will be reported to the veterinary staff to determine if treatment/support
  is appropriate and possible.
- Clinical or behavioral signs unresponsive to appropriate intervention within 24
  hours.
  - inactivity (decreased movement about the cage)
  - labored breathing
  - sunken eyes, squinting
  - hunched posture
  - intractable diarrhea
  - hemorrhage from a orifice
  - self-mutilation
  - failure to right itself when placed on side
  - neurologic signs (circling, ataxia)
  - piloerection/matted fur
  - progressive ulcerative dermatitis
  - one or more un-resolving skin ulcers
  - abnormal vocalization when handled
- tumors that affect normal function or that become ulcerated
- anorexia

**Non-Human Primates**
- Humane endpoints are determined by the veterinarian(s) in conjunction with the Language Research Center (LRC) director as studies conducted at the LRC are not invasive, therefore the studies are not expected to have any adverse effects on the animals.

**Birds**
- Loss of 20% of body weight from baseline weight when assigned to the protocol. If protocol is utilizing a young growing animal, a growth nomogram must be used to adjust the 20% weight deviation from a basal weight growing animals.
- Clinical or behavioral signs unresponsive to appropriate intervention within 24 hours.
  - inactivity (decreased flying about the cage)
  - labored breathing
  - sunken eyes, squinting
  - crouched posture
  - fluffed feathers
  - shaking
  - self-mutilation
  - failure to right itself when placed on side
  - neurologic signs (circling, ataxia)
  - abnormal vocalization when handled
  - anorexia
  - hemorrhage from a orifice

**Frogs**
- Clinical or behavioral signs unresponsive to appropriate intervention within 24 hours.
  - inactivity (decreased swimming around the tank)
  - anorexia
  - lack of response to stimuli
  - failure to right itself
  - neurologic signs (circling, ataxia)
- Surgical complications unresponsive to immediate intervention; i.e. bleeding, circulation failure, infection, and wound dehiscence.

**Lizards**
- Clinical or behavioral signs unresponsive to appropriate intervention within 24 hours.
  - inactivity (decreased movement around the tank)
  - anorexia
  - abnormal posture
  - skin darkening to dark brown or black
  - lack of response to stimuli
  - failure to right itself when placed on side
  - neurologic signs (circling, ataxia)
• Surgical complications unresponsive to immediate intervention; i.e. bleeding, circulation failure, infection, and wound dehiscence.

Death as an Endpoint

The use of death as an endpoint in animal experiments is strongly discouraged. Legal, regulatory, and moral guidelines require that animal pain and distress be minimized. For these reasons, investigators are encouraged to administer euthanasia in death endpoint experiments prior to the actual death of the animal unless a compelling case can be made that experimental validity would be irrevocably compromised.

These objectives assume that investigators can differentiate between animals that are found morbid (i.e. affected with disease and illness), and those that are found moribund (i.e. in the state of dying).

The IACUC believes that the principal investigator and the DAR veterinarian can judge and should perform euthanasia on moribund animals. Their judgment should be based on their professional experience with the animal model used in the experimental protocol. They should professionally and objectively evaluate the signs of dying with the animal model and perform euthanasia accordingly.

Investigators are expected to justify experimental endpoints and to agree that they can judge and will perform euthanasia on animals found moribund in a particular protocol. Moreover, all investigators are expected to monitor experimental animals at least daily (including weekends and holidays) to achieve this objective.

If experimental death itself is the required endpoint, the investigator must first receive approval to conduct such studies by providing strong scientific justification to the IACUC. Inconvenience or increased costs alone are not justifiable reasons.

Federal law authorizes veterinary staff to euthanize animals in states of unauthorized, uncontrolled pain or distress. The principal investigator is strongly encouraged to work closely with the veterinary staff in cases where uncontrolled pain or distress may develop.

The IACUC requires the following for research proposals that include death as an endpoint:

• Written justification, including discussion, of alternative endpoints.
• Justification of the numbers of animals to be included.
• Justification for non-use of analgesics if this is so.
• At least twice daily monitoring once animals exhibit abnormal signs.
• Maintenance of written records of monitoring.

12. Monitoring Animal Numbers on Protocols

Ordering Animals

As stated in the Institutional Animal Care and Use Committee Guidebook, (second edition, 2002) “Animals should be obtained only from licensed dealers or other legal sources, and it is incumbent upon an institution to establish mechanisms to monitor and document the number of animals acquired and used in approved activities. This it best accomplished if animal purchases may be made only through the institution’s animal resource facility or other appropriately designated office. Once animals have been
acquired, they should be included in a tracking system.” Accordingly, all animal acquisitions must be made via the GSU Department of Animal Resources.

If animals are wild-caught, the total number of animals obtained must be reported to the GSU Department of Animal Resources. Researchers must be aware of the total number of animals remaining on the protocol prior to catching animals in the wild so as not to exceed the total number approved on the protocol.

**Breeding Animals in-House**

Tracking animal use becomes more complicated when investigators maintain breeding colonies. Animals born on site are counted against one’s approved animal numbers either at the time of weaning or when the animal is first used on the protocol, whichever comes first. Animals that are genotyped prior to weaning and then euthanized before weaning if they are not of the correct genotype do not count as “animals used.”

**Transferring Animals prior to or at Weaning**

Researchers may move animals prior to, or at the time of weaning from a breeding protocol to a research protocol by recording the animal as being weaned on the destination protocol on the “Researcher Activity Sheet” in the animal housing room. No record is needed on the originating protocol, as animals do not count until they are weaned unless they are used for research prior to weaning. Also, animals harvested from the dam prior to birth do not count as “animals used”, however this is typically a terminal procedure for the dam, and that animal does count against the protocol.

**Transferring Animals after Weaning**

As it relates to tracking animal use in association with IACUC-approved protocols, the IACUC is particularly concerned with assuring that the number of animals used does not exceed the number approved for use. Further, in regards to animals being transferred from one IACUC-approved protocol to another, the IACUC must assure that the previous use of an animal does not preclude the proposed use of the animal (e.g. avoiding overuse of the animal consistent with regulatory mandates and guidelines). For example, an animal that has previously undergone a major survival surgery is typically not eligible to undergo a major survival surgery on a subsequent protocol.

The Division of Animal Resources Director or Associate to the Director can approve the transfer of an animal from one IACUC-approved protocol to another IACUC-approved protocol so long as the following criteria are met:

- The cumulative use of the animal cannot exceed the level of invasiveness approved on the protocol to which the animal is being transferred.
  - For example, an animal having undergone 2 blood collections from a peripheral vein (e.g. USDA pain category C blood collections) can be transferred to another protocol which is approved for pain category C blood collections. However, if the total amount of blood collections allowed on the recipient protocol is 5, the animal would only be eligible for 3 more blood collections.

- No animal may be used for multiple major survival surgeries unless such was approved on a protocol as interrelated components of one project.
  - For example, a rat having received an ovariectomy on Protocol A may not be transferred to Protocol B to receive a brain cannulation unless Protocol
B has approval to do ovariectomy and cannulation on the same animal at two different time points (multiple major survival surgery approval).

Researchers must submit an Animal Transfer form through the DAR Research Portal, outlining the historic use of the animals being transferred, the originating and destination protocols, and plan of use for the animals on the destination protocol. Please note that this transfer cannot take place until it has been reviewed and approved using the above delineated mechanism.

Any animal transfer requests which do not meet the criteria outlined above require prior review and approval by the IACUC.

13. Tracking Animals Use in Association with IACUC-Approved Protocols

As stated in the *Institutional Animal Care and Use Committee Guidebook*, (second edition, 2002) “Animals should be obtained only from licensed dealers or other legal sources, and it is incumbent upon an institution to establish mechanisms to monitor and document the number of animals acquired and used in approved activities. This it best accomplished if animal purchases may be made only through the institution’s animal resource facility or other appropriately designated office. Once animals have been acquired, they should be included in a tracking system.”

All animal acquisitions must be made via the Georgia State University Department of Animal Resources. In addition, an Animal Transfer Form has been developed and is to be used when an investigator wishes to transfer animals from one IACUC-approved protocol to another. Please note that this transfer cannot take place until it has been reviewed and approved.

As it relates to tracking animal use in association with IACUC-approved protocols, the IACUC is particularly concerned with assuring that the number of animals used does not exceed the number approved for use. Further, in regards to animals being transferred from one IACUC-approved protocol to another, the IACUC must assure that the previous use of an animal does not preclude the proposed use of the animal (e.g. avoiding overuse of the animal consistent with regulatory mandates and guidelines). For example, an animal that has previously undergone a major survival surgery is typically not eligible to undergo a major survival surgery on a subsequent protocol.

Tracking animal use becomes more complicated when investigators maintain breeding colonies. Animals born on site are counted against one’s approved animal numbers either at the time of weaning or when the animal is first used on the protocol, whichever comes first. Animals that are genotyped prior to weaning and then euthanized before weaning if they are not of the correct genotype do not count as “animals used.” Animals harvested from the dam prior to birth do not count as “animals used.” Should your protocol involve either the use of post-natal animals prior to weaning or the in-house production and weaning of animals, it is necessary to record this information on the Daily Census Sheet found in the animal housing room. The Department of Animal Resources will provide training on the use of this census sheet.

The Division of Animal Resources Director or Senior Administrative Coordinator in consultation with the IACUC Assistant Director can approve the transfer of an animal from one IACUC-approved protocol to another IACUC-approved protocol so long as the following criteria are met: the cumulative use of the animal cannot exceed the level of invasiveness approved on the protocol to which the animal is being transferred. For example, an animal having undergone 2 blood collections from a peripheral vein (e.g. USDA pain category C blood collections) can be transferred to another protocol which is approved for pain category C blood collections. However, if the total amount of blood collections allowed on the recipient protocol is 5, the animal would only be eligible for 3 more blood collections. In keeping with this policy, no animal may be used for multiple major survival surgeries unless such was approved on a protocol as interrelated components of one project. For example, a rat having received an ovariectomy on Protocol A may not be transferred to Protocol B to receive a brain cannulation unless Protocol B has approval to do ovariectomy and cannulation on the same animal at two different time points (multiple major survival surgery approval). Any animal transfer requests which do not meet the criteria outlined above require prior review and approval by the IACUC.

F. Mechanism for Receipt and Review of Concerns Involving Care and Use of Animals at Georgia State University as Registered Via Public Complaints and by Employees or Students

Concerns or complaints regarding animal usage at Georgia State University should be first brought directly to the attention of the individuals involved whenever possible, followed by notification to the IACUC. Any individual may report concerns to the IO, IACUC Chair, Institutional Veterinarian, or any member of the IACUC. They may also report concerns anonymously via the “Institutional Animal Care and Use Committee Anonymous Email Form” which is on the IACUC main web page. (https://ursadev.GSU.edu/forms/form.aspx?type=IACUC)

Notices are posted in the animal facilities advising individuals how and where to report animal welfare concerns and stating that any individual who, in good faith, reports an animal welfare concern will be protected against reprisals. Animal Welfare concerns are also discussed during the facility orientation.

Depending on the severity of the animal welfare concern, the Chair will either handle the issue administratively or will assign an ad hoc investigative committee after the entire IACUC has been informed of the concern. The ad hoc committee will immediately investigate the concern or complaint and report back to the IACUC. The individuals at whom the concern/complaint is directed will be informed of the nature of the concern/complaint and of the investigative procedures to be followed and given an opportunity to explain their side of the issue. As much documentation as is reasonably needed to support or refute concerns involving care and use of animals will be collected. Such information may include, but not necessarily be limited to, interviews of all parties involved, inspecting facilities, collection of pertinent documents, on-site evaluation of animals, and detailed review of procedures with responsible personnel. The full
IACUC will determine what action will be taken and immediately notify the principal investigator of such action. Reported concerns and all associated IACUC actions will be recorded in the IACUC meeting minutes. If an emergency situation exists, a DAR veterinarian should be contacted immediately. The veterinarian will take the necessary immediate action and report such action to the IACUC.

A written reply to those primarily involved will follow each written concern or complaint submitted to the IACUC. The Vice President of Research (the IO) will be notified in writing of significant and/or continuing animal welfare concerns. The IACUC, through the Institutional Official, shall file a report with appropriate federal or state agencies as dictated by the actions taken by IACUC and by applicable compliance standards for significant and/or continuing concerns. The Committee will report such actions to the IO and, as warranted, to OLAW.

No Georgia State University faculty, staff or student will be discriminated against, or be subject to any reprisal, for reporting noncompliance with any of the regulations or policies pertaining to animal care and treatment. Georgia State University has applicable whistleblower policies in place to protect individuals from reprisals for reporting animal welfare concerns. The online training module that is mandatory for any individual involved with research using animals discusses this legally required reporting mechanism. In addition, the reporting policy is posted inside each animal facility.

G. Euthanasia

Euthanasia literally means a “good death.” A more appropriate definition is a “gentle death.”

The USDA Animal Welfare Act defines euthanasia as “the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death.”

The Guide definition, “Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the AVMA Guidelines on Euthanasia (AVMA 2007 or later editions). In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; irreversibility; time required to induce unconsciousness; appropriateness for the species and age of the animal; compatibility with research objectives; and the safety of and emotional effect on personnel”. When it is necessary to euthanize animals as part of experimental protocols, it is very important to use appropriate euthanasia techniques.

Training

Because improper technique can cause pain and suffering to animals during euthanasia, you must be trained to properly and humanely perform euthanasia.

Do not perform euthanasia or any other procedure on an animal until a person experienced with the procedure has trained you and you feel confident performing the technique.

Verifying Death

It is very important that you make sure an animal is really dead before placing it in a bag and disposing of the bag. It is easy to mistake a deeply anesthetized animal for a dead animal, and
you do not want the animal to experience the terror of waking up in a closed bag and slowly suffocating to death. Therefore it is required that an additional method of insuring death, such as cervical dislocation, decapitation or thoracotomy, be employed to insure death after euthanasia by carbon dioxide overdose.

OLAW and USDA emphasize the importance of ensuring that euthanized animals are really dead, and further state that unintended recovery of animals after euthanasia represents 1) serious noncompliance with the PHS Policy and 2) a serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. Such incidents must be reported to OLAW by the IACUC with a full explanation of the circumstances and actions taken to prevent recurrence.

1. Methods of Euthanasia

PHS Policy and the Guide state that methods of euthanasia should be consistent with the most recent recommendations of a panel sponsored by the American Veterinary Medical Association, unless the IACUC approves deviations for scientific reasons. This Report of the AVMA Panel on Euthanasia https://www.avma.org/KB/Policies/Documents/euthanasia.pdf contains many guidelines used by the IACUC to evaluate methods of euthanasia.

Euthanasia methods can be broadly separated into physical and nonphysical (or pharmacologic) methods.

a. Physical methods

Physical methods rely on trauma to the head or spine to cause rapid death, or death due to fatal loss of blood. Examples include cervical dislocation, decapitation, captive bolt pistols, and exsanguinations ("bleeding an animal out").

b. Non-physical or pharmacologic methods

Non-physical or pharmacologic methods rely on drugs to cause loss of consciousness and death.

2. Hierarchy of Euthanasia Techniques

The various guidelines set up a hierarchy of euthanasia techniques, from most desirable to least desirable:

- Most desirable are nonphysical methods of euthanasia such as carbon dioxide inhalation and barbiturate overdose.
- Next are physical methods used in conjunction with sedation or anesthesia. Examples include exsanguination, decapitation, or cervical dislocation of an anesthetized animal.
- Less desirable are physical methods alone. Examples include exsanguination, decapitation, or cervical dislocation on a conscious animal without sedation or anesthesia. Such methods should not be used unless approved by the IACUC based upon scientific justification.
- Least desirable are methods of euthanasia disapproved by the Panel. Only under the most exceptional circumstances will the IACUC approve these methods.

Disapproved Methods

Several injectable agents are condemned by the AVMA Panel on Euthanasia as not appropriate when used alone. They include strychnine, nicotine, caffeine, magnesium sulfate, potassium chloride, and all neuromuscular blocking agents.
In addition, the following methods of euthanasia should not be used alone without special justification and IACUC approval:

b. Intra-cardiac Injections
Administration of injectable euthanasia agents into the heart provides rapid loss of consciousness and death. Intra-cardiac injections, however, should only be performed in heavily sedated, anesthetized, or comatose animals, unless the IACUC approves it after considering an extraordinary justification. The same holds true for blood collection from the heart.

c. Decapitation
There are two especially important issues regarding euthanasia of rodents and small rabbits.
The first is the use of decapitation alone. The primary justification for using decapitation without sedation or anesthesia is the need to recover tissues and body fluids that are chemically uncontaminated by sedatives or anesthetic agents or endpoint measures that are affected by sedatives/anesthetics. Special commercial guillotines designed to accomplish decapitation in a uniformly instantaneous manner are available.

The advantages of decapitation are that it may induce rapid unconsciousness, it does not chemically contaminate tissues, and it is rapidly accomplished thereby not altering many physiological parameters that would be affected by a slower death. The disadvantages are that the handling and restraint required to perform this technique may be distressful to animals, the guillotine blade is a hazard to personnel performing the technique, the technique may be aesthetically displeasing to personnel, and there is some experimental evidence that brain activity and sensory capabilities do not end immediately.

Consequently, the use of decapitation without prior anesthesia or sedation should be used in research settings only when scientifically justified by the user and approved by the IACUC.

d. Cervical Dislocation
Cervical dislocation is used to euthanize poultry, other small birds, mice, and immature rats and rabbits. Because the ligaments holding vertebrae together are too strong in larger animals to allow effective physical separation, cervical dislocation should only be performed on:

- Mice and small birds.
- Rats weighing less than 200 grams.
- Rabbits weighing less than 1 kg.

Remember that cervical dislocation is a physical method, and you should anesthetize or sedate the animals first, unless there are scientific reasons for not doing so approved by the IACUC. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.
For immature rabbits, the head is held in one hand and the hind limbs in the other. The animal is stretched and the neck is hyper-extended and dorsally twisted to separate the first cervical vertebra from the skull.

**Cervical Dislocation Advantages and Disadvantages**

**Advantages** of cervical dislocation are that it may induce rapid unconsciousness, it does not chemically contaminate tissue, and it is rapidly accomplished.

**Disadvantages** are that it may be aesthetically displeasing to personnel and that there is some experimental evidence that brain activity and sensory capabilities do not end immediately after dislocation.

e. **Exsanguination**

Exsanguination, or the near-complete withdrawal of blood from an animal, can be used to ensure death in unconscious animals. Because anxiety is associated with very low blood pressure, exsanguination should not be used as a sole means of euthanasia.

f. **Carbon Dioxide Inhalation**

Carbon dioxide inhalation is an effective means of euthanizing adult rodents. Bottled, compressed carbon dioxide is the only recommended source of the gas because the rate of inflow into the euthanasia chamber can be regulated precisely. Important points:

- Do not perform euthanasia or any other procedure on an animal until a person experienced with the procedure has trained you and you feel confident performing the technique.
- Use of carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g. antacids) is unacceptable.
- Carbon dioxide is not recommended for euthanasia on larger animals such as rabbits, dogs, cats, and swine because it appears to induce greater distress in these species.
- Only one species at a time should be placed into a chamber, and the chamber must not be overcrowded. When placed into the chamber, all animals must have floor space. Euthanasia should always be done in cohorts (live animals should not be placed in the chamber with dead animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.
- Because inspiration of high concentrations of CO₂ is both aversive and painful, in lieu of pre-charging the chamber, the animals should first be placed into the chamber, followed by the addition of CO₂ at a low flow rate (e.g. a rate sufficient to displace approximately 20% of the chamber volume per minute) to complete the process.
- As it is easy to mistake a deeply anesthetized animal for a dead animal, additional methods should be employed to ensure that death has occurred. Accordingly, gas flow should be maintained for at least 1 minute after apparent clinical death (e.g. at least one minute after the animal has quit breathing). Upon removal of the animal from the chamber, unintended
recovery must be obviated by the use of a physical method of euthanasia (e.g. thoracotomy, cervical dislocation, decapitation, or aortic transection).

- As neonatal rodents are resistant to high CO₂ levels, rodents under 11 days old should not be euthanized by carbon dioxide inhalation. Instead, rodent pups should be swiftly decapitated with a sharp pair of scissors.

**g. Isoflurane Euthanasia (using a drop jar)**

- Do not perform euthanasia or any other procedure on an animal until a person experienced with the procedure has trained you and you feel confident performing the technique.

- Only one species at a time should be placed into the drop jar, and the drop jar must not be overcrowded. When placed into the drop jar, all animals must have floor space. Euthanasia should always be done in cohorts (awake animals must not be placed in the chamber with anesthetized animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.

- This drop jar procedure must take place under a fume hood to prevent exposure of personnel to the isoflurane anesthetic. Please note that biosafety cabinets and animal transfer stations are not appropriate for this use as they will recirculate the isoflurane gas into the room.

- One should place isoflurane onto a cotton ball or gauze pad and establish a mechanism whereby the animal is unable to have direct contact with the cotton ball or gauze pad (an easy way to do this is to place the ball or pad into a tissue cassette).

- Once the animal is anesthetized (no spontaneous movement apart from respirations, no foot withdrawal in response to toe pinches as assessed on all four feet), remove the animal from the drop jar and immediately proceed to perform a secondary method of euthanasia (e.g. bilateral thoracotomy, cervical dislocation, decapitation, or aortic transection). It is imperative that the secondary method of euthanasia be done quickly as the animal, once removed from the drop jar, is able to regain consciousness quickly. Accordingly, only one animal at a time may be removed from the drop jar.

**h. Isoflurane Euthanasia (using a precision vaporizer)**

- Do not perform euthanasia or any other procedure on an animal until a person experienced with the procedure has trained you and you feel confident performing the technique.

- Only one species at a time should be placed into a chamber, and the chamber must not be overcrowded. When placed into the chamber, all animals must have floor space. Euthanasia should always be done in cohorts (awake animals must not be placed in the chamber with anesthetized animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.

- Induce anesthesia in the induction chamber utilizing 3-5% isoflurane (follow the anesthesia machine directions affixed to the anesthesia machine for proper oxygen flow rates, etc.).

- Once the animal is anesthetized (no spontaneous movement apart from respirations, no foot withdrawal in response to toe pinches as assessed on all four feet), remove the animal from the induction chamber and place on the nosecone to maintain the anesthetized state and then perform a physical
method of euthanasia (e.g. bilateral thoracotomy, cervical dislocation, decapitation, or aortic transection).

- After use, turn off the isoflurane and oxygen and disinfect the anesthetic machine per the DAR SOP posted in the room.

i. **Pithing**

Pithing is the destruction of the central nervous system by mechanical means. The brain, the spinal cord, or both may be destroyed, depending on the species and additional methods of euthanasia used. Pithing is a physical means of euthanasia, and thus should be used only if nonphysical methods are not appropriate.

Accordingly, pithing is generally used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species such as frogs, with anatomic features that facilitate easy access to the central nervous system, pithing may be used as a sole means of euthanasia, but anesthetic overdose is a more suitable method.

Pithing requires knowledge of anatomic landmarks and requires great skill. Like all methods of euthanasia, it should only be performed by trained personnel.

j. **Reducing Animal Anxiety during Euthanasia**

When animals are euthanized, other animals should not be immediately present because vocalization and release of pheromones in urine and feces can occur during euthanasia that induces anxiety in other animals. Similarly, euthanasia chambers should be cleaned well between uses to reduce animal anxiety caused by exposure to alarm pheromones in urine and feces.

k. **Weight Loss as an Endpoint**

**Immature animals:**

Maximum weight loss is a deviation of 20% from recognized growth curves or age-matched control animals. All protocols involving excessive weight loss will be evaluated on a case-by-case basis.

Background weight losses may occur in research animals in association with a variety of experimental regimens including studies where feed and essential nutrients are withheld, such as studies of nutritional deficiencies, toxicology or cancer. Weight loss also occurs in association with many spontaneous diseases and is a prime indicator of declining clinical condition. Moderate food restriction and weight loss, rather than being detrimental, has been shown to promote health and extend the life of laboratory rodents and other species.

The IACUC has accepted weight losses of greater than 20% that can be scientifically justified. The upper limit of acceptable weight loss in mature animals on experimental regimens shall generally be 20%. Written scientific justification must be provided to the IACUC for approval for a greater than 20% weight loss.

In studies where weight loss is expected to occur, monitoring must be done by investigative staff trained and experienced in recognizing clinical signs of illness and distress in study animals. Weights must also be taken at least weekly under such circumstances and be readily available for review by the veterinary staff and the IACUC.
In their protocol submissions, investigators must address situations where weight loss will exceed proposed limits and remedial measures that will be taken. Veterinary staff may intervene when such remedial measures prove ineffective or to address weight losses that occur in excess of 20% of pre-study body weight in any research animal, or when other limits approved by the IACUC have been reached or exceeded. Such intervention may include euthanasia. Exceptions to this policy will be allowed only if there is a veterinary determination that weight losses exceeding approved limits are not endangering animal health and well-being and a specific waiver is obtained from the IACUC.

H. Animal Surgery
The Animal Welfare Act (AWA), Public Health Service (PHS) Policy, and the “Guide for the Care and Use of Laboratory Animals” (Guide) require the Institutional Animal Care and Use Committee (IACUC) and a DAR veterinarian to provide oversight of all surgical procedures. The Guide states that, “Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. Regardless of species, aseptic technique includes preparation of the patient, such as hair or feather removal and disinfection of the operative site; preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile surgical gloves; sterilization of instruments, supplies, and implanted materials; and the use of operative techniques to reduce the likelihood of infection.”

1. Sterile or Aseptic Technique
This refers to a series of practices followed to prevent the contamination of the surgical site by microbes during surgery. If an animal will recover from surgery, sterile technique must be used.

2. General Anesthesia
General anesthesia is a state of unconsciousness characterized by a complete lack of pain and sensory perception.

3. Regional / Local Anesthesia
In contrast to general anesthesia, regional (or local) anesthesia refers to preventing pain and sensory perception in one small part or a region of the body, such as a section of skin or an entire limb. Before beginning surgery, you must ensure that the animal will not feel pain during the procedure. General or regional anesthesia must be provided.

4. Anesthesia and Analgesia
One must administer appropriate anesthesia and or analgesia to animals undergoing procedures that cause more than momentary or slight pain or distress, unless scientifically justified and approved by the Institutional Animal Care and Use Committee (IACUC). Investigators are always strongly encouraged to consult with the DAR veterinarians in the design of their animal research projects, including the selection of the most efficacious anesthetic and analgesic regimens for the animals and the model.

5. Anesthetics
Anesthetics render the animal unconscious without loss of vital functions. It is important to provide appropriate and gentle restraint, a sufficient amount of analgesia to diminish pain sensation during the procedure, and relaxation of muscle tone to the degree that procedures can be performed quickly and efficiently.
• Inhalant anesthetics (i.e., isoflurane) – Provide a safe, reliable, reversible, and reproducible means of rendering an animal unconscious to perform surgeries or other procedures that require the animal to be unconscious. Adjusting the inhaled percentage of anesthetic gas to deepen anesthesia is far safer than repeated re-dosing of injected drugs. Volatile anesthetics are easier to decrease as well, even compared to drugs for which there is an injectable antagonist or reversal agent. Delivery of inhaled anesthetics for rodents is typically via an induction box for the induction of anesthesia and via a nosecone for the maintenance of anesthesia. In some cases, endotracheal intubation may be utilized for anesthetic maintenance in lieu of a nosecone. A disadvantage of the inhalant anesthetic agents is the lack of residual analgesia once the vaporizer has been turned off as the animals will recover quickly from the anesthetic state. However, the quick recovery also constitutes an advantage in that the animal can regain its normal thermoregulatory status much faster as compared to injectable anesthetics (hypothermia has the potential to be a significant cause of mortality in anesthetized rodents). The use of pre-emptive analgesia is necessary.

• Injectable anesthetics (i.e., ketamine combinations) – Injectable anesthetics are appropriate for some procedures. There is, however, a great deal of variation in depth and duration of anesthesia among rodent strains and individual animals. When using injectable anesthetics it is important to consider accurate dosing with correct multidrug use ratios, storage conditions, and feasibility of immediate use following reconstitution. It is critical to weigh each animal accurately prior to administration of a calculated dose of anesthesia to avoid either over- or under-dosing.

• Local anesthetics (i.e., lidocaine, ethyl chloride spray) – Local anesthetics can be injected or applied topically at the site of the incision and may be appropriate to consider as supplements to either inhalant or injectable anesthetics.

**Inhalant Anesthetic**

<table>
<thead>
<tr>
<th>Species</th>
<th>Induction dose</th>
<th>Maintenance dose</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>4-5%</td>
<td>1-2%</td>
<td>Inhaled (Induction box and nose cone)</td>
</tr>
<tr>
<td>Rats</td>
<td>4-5%</td>
<td>1-2%</td>
<td>Inhaled (Induction box and nose cone)</td>
</tr>
<tr>
<td>Hamsters</td>
<td>4-5%</td>
<td>1-2%</td>
<td>Inhaled (Induction box and nose cone)</td>
</tr>
</tbody>
</table>

**Injectable Anesthetic**

<table>
<thead>
<tr>
<th>Species</th>
<th>Mice (mg/kg, unless otherwise noted)</th>
<th>Rats (mg/kg, unless otherwise noted)</th>
<th>Syrian Hamsters (mg/kg, unless otherwise noted)</th>
<th>Siberian Hamsters (mg/kg, unless otherwise noted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlora hydrate</td>
<td>370-400 IP</td>
<td>300-450 IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine/Diazepam</td>
<td>100 K/5 D IP</td>
<td>40 K/5 D IP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ketamine/Midazolam  50-75 K/1-10 M IP  60 K/0.4 M IP
Ketamine/Xylazine  90-150 K/7.5-16 X IP  40-80 K/5-10 X IP, IM  150-200 K/10 X IP  50-100 K/5-10 X IP
Ketamine/Xylazine/Acepromazine  100 K/2.5 X/2.5 A IM  40 K/8.0 X/4.0 A IM
Sodium pentobarbital  30-90 IP  30-60 IP
Thiobarbital (Inactin)  80 IP
Tiletamine+Zolazepam (Telazol)  20-40 IP
Tiletamine+Zolazepam (Telazol)/Xylazine  30 T/10 X IP  30 T/10 X IP
Reverse Xylazine with Yohimbine  2.1 IP

*IP = intraperitoneal. IM = intramuscular. SC = subcutaneous. IV = intravenous.
1 For use in terminal procedures only.

### Local or Topical Anesthetic

<table>
<thead>
<tr>
<th></th>
<th>Mice (mg/kg, unless otherwise stated)</th>
<th>Rats (mg/kg, unless otherwise stated)</th>
<th>Syrian Hamsters (mg/kg, unless otherwise stated)</th>
<th>Siberian Hamsters (mg/kg, unless otherwise stated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupivacaine 0.25-0.5% / Lidocaine 1-2%</td>
<td>50:50 bupivacaine / lidocaine* Local infiltration SC</td>
<td>50:50 bupivacaine / lidocaine* Local infiltration SC</td>
<td>50:50 bupivacaine / lidocaine* Local infiltration SC</td>
<td>50:50 bupivacaine / lidocaine* Local infiltration SC</td>
</tr>
<tr>
<td>Ethyl chloride spray</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
</tr>
<tr>
<td>Cold ethanol</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
</tr>
<tr>
<td>Lidocaine/prilocaine cream (EMLA ream®)</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
<td>Local application Topical</td>
</tr>
</tbody>
</table>

*Prepare a 50:50 volume mixture of Bupivacaine 0.25-0.5% / Lidocaine 1-2%. Infiltrate the incision area subcutaneously prior to making the incision. Lidocaine provides almost immediate pain control with a duration of 20-40 minutes and bupivacaine provides longer pain control (lasting 4–6 hours). Lidocaine/bupivacaine should be administered after the animal is anesthetized. Lidocaine and bupivacaine should not exceed 10 and 6 mg/kg respectively.

6. **Analgesics**

Improved pain management for animals is an important goal in the use of experimental animals. One must administer analgesia to animals undergoing procedures that cause more than momentary or slight pain or distress unless scientific justification has been provided and approved by the IACUC for withholding such medications. Analgesics reduce or relieve pain without loss of consciousness. The avoidance or minimization of
discomfort, distress and pain in laboratory animals is a moral imperative for all individuals who work with these species in biomedical research.

Systemic and/or local analgesics may also reduce the anesthetic requirements, and have a pre-emptive effect on pain perception which persists into the recovery period. Analgesics should be administered pre-operatively as well as post-operatively for adequate pain relief in post-surgical animals.

The most commonly used analgesics in laboratory animal species are opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). The ultimate decision for selection of drug must be based upon the experimental model under study and the specific types of data to be collected. The investigator is encouraged to consult a Division of Animal Resources (DAR) veterinarian for guidance on appropriate analgesics for the species being used and the procedures being performed.

- Opioids (i.e., buprenorphine, morphine) – Opioids are very effective analgesics for surgical pain but may have effects on cardiovascular function and can be sedating.
- Non-steroidal anti-inflammatory agents (i.e., meloxicam, carprofen) – Some non-steroidal anti-inflammatory analgesics (NSAIDs) may have longer durations of action than most available opioids. These drugs are frequently co-administered with an opioid to combine potency of effect with duration of action.

<table>
<thead>
<tr>
<th>Analgesics</th>
<th>Mice (mg/kg, unless otherwise stated)</th>
<th>Rats (mg/kg, unless otherwise stated)</th>
<th>Syrian Hamsters (mg/kg, unless otherwise stated)</th>
<th>Siberian Hamsters (mg/kg, unless otherwise stated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>300/day in drinking water</td>
<td>300/day in drinking water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.05-0.1 every 12 hr SC</td>
<td>0.05-0.5 every 12 hr SC</td>
<td>0.5 every 8 hr SC</td>
<td>0.5 every 8 hr SC</td>
</tr>
<tr>
<td>Buprenorphine SR1</td>
<td>0.05-0.1 every 48-72 hr SC</td>
<td>1.0-1.2 every 48-72 hr SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butorphanol</td>
<td>5 every 4-6 hr SC</td>
<td>2 every 4-6 hr SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carprofen (Rimadyl)</td>
<td>5 every 12 hr SC</td>
<td>5 every 12 hr SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flunixin meglumine (Banamine)</td>
<td>2.5 BID SC</td>
<td>2.5 BID SC</td>
<td>2.5 BID SC</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>40/day in drinking water</td>
<td>15/day in drinking water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoprofen (Ketofen)</td>
<td>2-5 every 12-24 hr SC</td>
<td>2-5 every 12-24 hr SC</td>
<td>5 SID SC</td>
<td>5 SID SC</td>
</tr>
<tr>
<td>Meloxicam2</td>
<td>1 SID SC, PO</td>
<td>1 SID SC, PO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meperidine</td>
<td>10-20 every 2-3 hr SC</td>
<td>5-10 every 2-3 hr SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>10 every 2-4 hr</td>
<td>10 every 2-4 hr</td>
<td>80 every 2-4 hr</td>
<td>80 every 2-4 hr</td>
</tr>
</tbody>
</table>

56
<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>SC</th>
<th>SC</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalbuphine</td>
<td>4-8 every 4-8 hr SC</td>
<td>1-2 every 4-5 hr IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>0.1 every 4 hr SC</td>
<td>0.3 every 4 hr SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylazine</td>
<td>5-12 every 2 hr SC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reversal for Xylazine is Yohimbine</td>
<td></td>
<td>2.1 IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reversal for Opioid³ is Naloxone</td>
<td>0.05-0.1 IP, IV</td>
<td>0.01-0.1 IP, IV</td>
<td>0.01-0.1 IP, IV</td>
<td></td>
</tr>
</tbody>
</table>

¹Buprenorphine SR has been reported to cause skin reactions at the injection site. Please consult DAR veterinarians for direction on administration prior to use.
²In mice, it is recommended to dilute injectable meloxicam to a concentration of 0.5 mg/ml.
³Naloxone is used as a reversal agent for opioids specially morphine, buprenorphine, it should not be used to reverse butorphanol.

*SID = once a day. BID = twice a day.
*IP = intraperitoneal. IM = intramuscular. SC = subcutaneous. IV = intravenous.

7. **Supportive care**

Non-pharmaceutical methods to support the animal during the administration of anesthetic and analgesic agents should be used and include:
- Keeping the animal warm during and after anesthetic procedures
- Fluid administration (if applicable)
- Keeping recovering animals isolated in a quiet area
- Providing supplemental foods (if applicable)

8. **Monitoring**

Plans for intra- and post-operative monitoring must be included in the IACUC protocol. Monitoring the anesthetized animal includes assessing responsiveness to painful stimuli (i.e. pinching a foot, surgical manipulation), assessing the character of respirations (i.e. increase respirations means the animal is too light or decrease respirations means the animal is too deep), and assessing the skin or mucous membrane color for a pink color, seen by observing the ears, tail, and oral mucosa or foot pads.

Pedal withdrawal reflex (footpad-pincho) is recommended for assuring adequate depth of anesthesia prior to first incision and as a repeated check throughout the procedure. Depending on the procedure, other monitoring may be indicated such as the respiratory rate. Monitoring should be recorded through the post-operative period to complete recovery. As a general rule animals should be observed every 15 minutes.

9. **Controlled Substances**

Several commonly used anesthetics and analgesics (i.e., ketamine, opioids) are controlled substances by the Drug Enforcement Administration (DEA) and, as such, require the investigator to be registered with both the DEA and the Georgia Board of
Pharmacy prior to the use of such substances. Controlled substances carry special
storage and record keeping requirements. Provided is the link for the GSU Controlled
Substance Policy, http://ursa.research.gsu.edu/files/2013/11/Controlled-Substances-
Policy.pdf.

10. Mixing or Diluting Anesthetic/Analgesic Agents

- A sterile diluent should be used (e.g. sterile physiological saline)
- The substance should be stored in a sterile injection vial
- The injection vial must indicate the name of the substance(s), the concentration, and
  the date of expiration. Regarding date of expiration, please note the following: Single
  compounds: The date listed on the original bottle or box; Mixtures/Compounds: The
  earliest date listed on any agent which is mixed or compounded;
- Multiple-dose injectable vials should not be used if they contain particulate matter,
  precipitates, turbidity, or discoloration.
- Sterile fluids (with no drugs added after opening) that intended to be accessed
  multiple times to obtain small volumes for administration and drug mixing can be
  used for 30 days beyond initial opening if containers are kept refrigerated and
  accessed in an aseptic manner.

11. Anesthetic Machine Maintenance

As a component of maintaining properly functioning anesthetic machines, such
machines should receive an inspection on a regular basis (at least once every two
years) by a qualified service technician. Such an individual is able to check for leaks,
replace worn parts, and check the calibration of the vaporizer. Vaporizers must be
serviced and/or calibrated if the delivery of anesthetic gas is not within 20% of the
metered concentration. The service technician will place documentation of the biannual
inspection on each anesthetic machine inspected.

A properly functioning anesthetic machine thereby reduces the likelihood of
environmental contamination of anesthetic gases and helps to ensure that the animal is
receiving the desired concentration of anesthetic.

In addition, any anesthetic machine showing signs of wear or other problems must be
removed from service immediately until appropriate repairs are rendered. Such signs of
wear or other problems include the following: discoloration (yellowish-brown) in the “fill”
sight glass of the vaporizer, cracked or damaged hoses, sticking valves or knobs, and/or
animals not responding as anticipated to the level of anesthesia provided.

For personnel and animal safety and well-being, all individuals using inhalant anesthetic
machines should check for leaks prior to each use of the machine. Further, such
individuals should assure that the activated charcoal scavenger canister associated with
the machine has not exceeded the allotted weight gain (see below).

Activated charcoal scavenger canisters (e.g. F/Air canisters) are only able to adsorb a
specified amount of anesthetic gas after which time the canister is “spent” and must be
replaced. Scavenger canisters must be weighed prior to being put into use and the
starting weight recorded. Scavenger canisters should be weighed often enough so that
they are always at a functioning weight (e.g. F/Air canisters should be disposed of after a
50 gram increase in weight – other scavenger canister brands may allow for a larger
weight increase prior to disposal – follow directions on the canister itself). Because only
a few spots for weight recording are provided on the canisters, a log may be kept for each canister. When canisters reach their disposal weight, they can be disposed of by contacting the GSU Biosafety Officer to arrange for pickup.

12. Survival Surgery
Survival surgery is surgery in which the animal regains consciousness after anesthesia. If an animal undergoes survival surgery, aseptic (sterile) technique must be used to prevent postoperative infections, no matter what vertebrate species is involved. The incision site must be properly prepared before the incision. The hair must be clipped and the skin must be disinfected, often with chlorhexidine or iodine solutions. Drugs and supplies intended to be used in animals must be in-date.

a. Aseptic Technique for Animal Surgery
The Animal Welfare Act (AWA), Public Health Service (PHS) Policy, and the “Guide for the Care and Use of Laboratory Animals” (Guide) require the Institutional Animal Care and Use Committee (IACUC) and a DAR veterinarian to provide oversight of all surgical procedures. The Guide states that, “Aseptic technique is used to reduce microbial contamination to the lowest possible practical level. Regardless of species, aseptic technique includes preparation of the patient, such as hair or feather removal and disinfection of the operative site; preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile surgical gloves; sterilization of instruments, supplies, and implanted materials; and the use of operative techniques to reduce the likelihood of infection.”

Minimum aseptic technique for rodent and avian survival surgery:

Surgical area:

i. Any dedicated space in an investigator’s laboratory appropriately managed to minimize contamination from other activities in the room. A procedure room in the animal facility can also be utilized. The area must be uncluttered and disinfected prior to the surgical procedure.

ii. No other activities can be performed in this same area while surgery is being performed.

iii. Patient preparation (e.g. hair clipping and the disinfection of clipped area) must be conducted in a location separate from the surgical site (the separation can either be a separate room or a different location in the same room).

Surgical instruments and supplies:

i. Sterile instruments and supplies are required.

ii. Instruments are initially autoclaved or gas sterilized. Alternatively, chemical sterilizing agents may be used for instruments and implantable devices consistent with manufacturer’s recommendations regarding exposure times necessary to achieve sterilization and subsequent washing procedures (e.g. with sterile water or saline) to remove the chemical sterilant prior to use of the item.
iii. If performing batch surgeries on rodents (e.g. using the same instruments on a series of animals during one surgical session) one must wipe the instruments clean (e.g. with sterile saline and sterile gauze) to remove gross contamination and re-sterilize the instrument tips (e.g. in a hot bead sterilizer between animals) before re-use.

iv. Again, if performing batch surgeries, one must ensure that the gloves remain sterile between surgeries. If sterility was broken between animals new gloves must be donned. If sterility was not broken or if the tips-only technique is being utilized (see below) the same gloves can be used on the subsequent animal provided that the gloves are wiped clean of any blood between animals with a sterile physiological fluid (e.g. sterile physiological saline).

**Surgeon preparation:**

i. Hand scrubbing and rinsing of all hand surfaces

ii. Gloves (either sterile surgical gloves OR clean exam gloves, see below)
   - Sterile surgical gloves. Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.
   - Clean exam gloves. Using clean exam gloves and a “tips-only” technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, the interior tissues of the animal, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, stereotactic apparatus, and lighting) is required.

iii. A surgeon's mask

iv. A lab coat, surgical gown, and/or dedicated surgical scrub top.

**Patient preparation:**

i. Clipping of fur (or use of a depilatory cream) or removal of feathers over the patient’s surgical site; this is to occur in a location that is removed from the surgery area (another room may be utilized or a different location on a lab bench also meets this objective). Loose hair may be removed by touching the prepared area with the sticky side of tape or by careful vacuuming.

ii. Preparation of surgical site by scrubbing the skin with a disinfectant (e.g., chlorhexidine, betadine) followed by 70% alcohol or sterile water. The area is scrubbed with a new clean gauze pad or sterile cotton swab in a gradually enlarging circular pattern from the center of the proposed incision to the periphery. The gauze pad or swab should not be brought back from the contaminated
periphery to the clean central area. This process is completed 3 times.

iii. Lubricating ophthalmic ointment (such as Lacrilube or Tearfair) must be placed in the anesthetized animal's eyes to prevent drying of the cornea if the anesthesia and recovery will last more than five minutes.

iv. To prevent hypothermia, an attempt should be made not to wet the animal any more than necessary and the animals should be placed on a heating pad (using a circulating water blanket, warm water bottle, bubble wrap, or equivalent external heat source, taking care to not cause thermal burns to the animal's skin). Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal.

Surgical draping of the disinfected area is required when sterile tissues or instrument may come in contact with non-sterile portions of the animal's body or other non-sterile surfaces.

i. Sterile surgical drapes are available for purchase commercially or one can autoclave a cloth drape or utilize autoclaved gauze for this purpose. Alternatively, the GSU IACUC accepts the use of an adhesive plastic food wrap (e.g. Press-n-Seal is the only currently available product), pulled from a clean roll and applied across the surgical field and surrounding area as an appropriate surgical drape for rodent surgeries. Please see the DAR veterinary staff for details; the incision can be made directly through the plastic wrap and into the skin.

Minimum aseptic technique for all other mammalian species (e.g. rabbit, ferret, and others) survival surgery:

Surgical area: According to The Guide, Major surgeries must be performed, “…in facilities intended for that purpose…” Practically, this means:

i. Interior surfaces are monolithic and impervious to moisture.

ii. Ventilation supply is filtered air at positive pressure. iii. There is minimal traffic.

iv. A surgeon preparation area is located outside of the operating room.

v. A patient preparation area is separate from the surgeon preparation area.

vi. A patient recovery area is provided.

Surgical instruments and supplies:

i. Sterile instruments and supplies are required.

ii. Instruments are initially autoclaved or gas sterilized. Alternatively, chemical sterilizing agents may be used for instruments and implantable devices consistent with manufacturer's recommendations regarding exposure times necessary to achieve sterilization and subsequent washing procedures (e.g. with sterile
water or saline) to remove the chemical sterilant prior to use of the item.
1. NOTE: The preference is for autoclave and gas sterilization.
2. NOTE: Alcohol is neither a sterilant nor a high-level disinfectant and is not appropriate for instrument preparation.
   
   iii. Appropriate indicators and verification must be located with instruments that have been sterilized.
   
   iv. Instruments which contact non-sterile materials or surfaces during the procedure must be discarded from the surgical field and not used until re-sterilized.

**Surgeon preparation:**

i. Hand scrubbing and rinsing of all hand surfaces
ii. Sterile surgeon’s gloves (not exam gloves)
iii. A surgeon’s mask
iv. A surgical gown or dedicated surgical scrubs
v. Hair bonnet

**Surgical site prep:**

i. Clipping of fur over the patient’s surgical site; this is to occur in a location that is removed from the surgery area (another room). Loose hair may be removed by touching the prepared area with the sticky side of tape or by careful vacuuming.

ii. Preparation of surgical site by scrubbing the skin with a disinfectant (e.g., chlorhexidine, betadine) followed by 70% alcohol or sterile water. The area is scrubbed with a new sterile surgical gauze pad in a gradually enlarging circular pattern from the center of the proposed incision to the periphery. The gauze pad should not be brought back from the contaminated periphery to the clean central area. This process is completed 3 times.

iii. Lubricating ophthalmic ointment (such as Lacrilube or Tearfair) must be placed in the anesthetized animal’s eyes to prevent drying of the cornea if the anesthesia and recovery will last more than five minutes.

iv. To prevent hypothermia, an attempt should be made not to wet the animal any more than necessary and the animals should be placed on a heating pad (e.g. using a circulating water blanket, taking care to not cause thermal burns to the animal's skin). Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal.

**Surgical draping** of the disinfected area is required when sterile tissues or instrument may come in contact with non-sterile portions of the animal’s body or other non-sterile surfaces.

**Minimum aseptic technique for fish and amphibian survival surgery:**

**Surgical area:**
i. Any dedicated space in an investigator's laboratory appropriately managed to minimize contamination from other activities in the room. A procedure room in the animal facility can also be utilized. The area must be uncluttered and disinfected prior to the surgical procedure.

ii. No other activities can be performed in this same area while surgery is being performed

**Surgical instruments and supplies:**

i. Sterile instruments and supplies are required.

ii. Instruments are initially autoclaved or gas sterilized. Alternatively, chemical sterilizing agents may be used for instruments and implantable devices consistent with manufacturer’s recommendations regarding exposure times necessary to achieve sterilization and subsequent washing procedures (e.g. with sterile water or saline) to remove the chemical sterilant prior to use of the item.

iii. If performing batch surgeries (e.g. using the same instruments on a series of animals during one surgical session) one must wipe the instruments clean (e.g. with sterile saline and sterile gauze) to remove gross contamination and re-sterilize the instrument tips (e.g. in a hot bead sterilizer between animals) before re-use.

iv. Again, if performing batch surgeries, one must ensure that the gloves remain sterile between surgeries. If sterility was broken between animals new gloves must be donned. If sterility was not broken or if the tips-only technique is being utilized (see below) the same gloves can be used on the subsequent animal provided that the gloves are wiped clean of any blood between animals with a sterile physiological fluid (e.g. sterile physiological saline).

**Surgeon preparation:**

i. Hand scrubbing and rinsing of all hand surfaces

ii. Gloves (either sterile surgical gloves OR clean exam gloves, see below)

   • Sterile surgical gloves. Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.

   • Clean exam gloves. Using clean exam gloves and a “tips-only” technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, the interior tissues of the animal, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, stereotactic apparatus, and lighting) is required.

iii. A surgeon’s mask
iv. A lab coat, surgical gown, and/or dedicated surgical scrub top.

Patient preparation:

i. Patient preparation in fish and amphibians should minimize disruption of the skin and natural mucus, because these are major barriers to infection. Thus, preparation of the patient consists of wiping the surgical site with a cotton swab soaked in sterile saline to reduce gross contamination in the area of surgery.

iii. Lubricating ophthalmic ointment (such as Lacrilube or Tearfair) must be placed in the anesthetized animal’s eyes to prevent drying of the cornea if the anesthesia and recovery will last more than five minutes.

iv. Surgical draping of the disinfected area is required when sterile tissues or instrument may come in contact with non-sterile portions of the animal’s body or other non-sterile surfaces.

Postoperative Care:

i. Recovering animals should not be placed onto loose bedding material until they are fully awake, as suffocation can result. A paper towel or similar material may be placed between the bedding and the animal until it awakens from anesthesia. Hypothermia may be prevented or treated by placing the recovering animals in a warm cage (e.g. as occurs when one places the cage on a supplemental heat source such as a circulating water pad). Be cautious with supplement heat sources; hyperthermia can be as detrimental as hypothermia.

ii. Dehydration can be ameliorated by the administration of appropriate fluid therapy. Initially this may be done by giving 1 to 2 ml of warm fluids (0.9% sterile NaCl or equivalent) per 100 g of body weight by subcutaneous or intraperitoneal injection. If blood loss occurred during the surgical procedure, or if the animal is slow to recover from anesthesia, provide additional fluids. Veterinary staff may be consulted for assistance with appropriate fluid therapy.

iii. Analgesia must be administered to control post-surgical pain as described in the IACUC approved protocol, unless there is approval by the IACUC not to provide analgesics. Analgesia should be administered before or during surgery for optimal effect. Veterinary staff may be consulted for appropriate analgesia regimens.

iv. Animals should not be returned to the animal housing room until they are sternal and clearly awake. To prevent cannibalism or suffocation, rodents should be housed individually until they are ambulatory.

v. Materials that have expiration dates:
13. **Non-survival surgery**
Non-survival surgery is surgery in which the animal is euthanized while under anesthesia, and does not regain consciousness. If an animal undergoes non-survival surgery, **sterile technique may not be required**. Even though the animal will not survive beyond the end of surgery, **at a minimum, the surgeon should wear gloves, the surgical site should be clipped, and the instruments and work area should be clean**. Expired products other than anesthetics or analgesics, may be used for non-survival procedures. Expired anesthetics or analgesics may not be used at any time. The Guide states the following “… at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. For non-survival procedures of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome.”

a. **Procedural techniques** which encourage proper surgical outcomes include:
   i. Gentle tissue handling
   ii. Minimal dissection of tissue
   iii. Appropriate use of instruments
   iv. Effective hemostasis
   v. Correct use of suture materials and patterns
   vi. Reducing surgical time
   vii. Minimum aseptic technique for all non-survival surgery:

14. **Major vs. Minor Surgery**
Surgical procedures are defined as major or minor by the IACUC and DAR veterinarians on a case-by-case basis. The following definitions will apply as a general guideline.

A. **Major survival surgery**: Any surgical intervention that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection.

B. **Minor survival surgery**: Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation through a skin incision, and the like.

Regardless of whether the surgeries are classified as major or minor, multiple survival surgical procedures must be evaluated by the IACUC to determine their impact on animal wellbeing. No animal assigned to a protocol is to be used in more than one major survival operative procedure unless:

1. The multiple procedures are included within one protocol;
2. The Principal Investigator provides scientific justification in the protocol; and
3. The Institutional Animal Care and Use Committee (IACUC) provides its approval.

15. **Multiple Major Survival Surgeries**
Multiple procedures that may induce substantial post-procedural pain or impairment may be conducted on a single animal only if justified by the PI, and reviewed and approved
by the IACUC. Multiple major surgical procedures on a single animal are acceptable only if they are:

- included in and essential components of a single research project or proposal;
- scientifically justified by investigator; or
- necessary for clinical reasons.

Cost saving alone is not an adequate reason for performing multiple major survival surgical procedures. Note that under USDA regulations (AWR 2.31 (x) A-C), “No animal will be used in more than one major operative procedure from which it is allowed to recover, unless: (A) Justified for scientific reasons by the principal investigator, in writing; (B) Required as routine veterinary procedure or to protect the health or well-being of the animal as determined by a DAR veterinarian; or (C) In other special circumstances as determined by the [Animal Care] Administrator on an individual basis. Written requests and supporting data should be sent to the Animal and Plant Health Inspection Service, Animal Care, 4700 River Road, Unit 84, Riverdale MD 20737-1234”.

OLAW’s guidance on use of multiple surgical procedures was first published in 1997 (Contemporary Topics, 1997; 36(2):47-50) and posted on the OLAW website on September 11, 2006. Major survival operative procedures cannot be performed a second time on an animal, even if it is transferred to separate IACUC proposal. Animals surviving a major operative procedure must be identified (written documentation) to prevent their use in a second major survival operative procedure.

16. Surgery Location

The rooms that can be used for surgery vary depending on:

- The species.
- Whether a surgery is major or minor.
- Whether the surgery is survival or non-survival. A dedicated surgical suite is required for major survival surgery on all non-rodent mammals (this includes rabbits). In contrast, a clean area or portion of a room (along with the use of aseptic technique) is acceptable for:
  - Major survival surgeries on rodents and lower vertebrates.
  - All non-survival surgeries.
  - All minor survival surgeries.

17. Postoperative Care for Survival Surgeries

Postoperative care must be provided after survival surgeries. The animal should be monitored to make sure it is recovering properly. If the surgical procedure would be expected to cause pain in a human, then it should be assumed that the procedure will be painful in an animal, no matter the species, and appropriate postoperative analgesia should be provided unless nonuse is approved by the IACUC. The agent, dose, route, frequency, and duration of postoperative analgesia provided should be discussed with and approved by a DAR veterinarian during the planning stages of the experiments.

a. Documenting Postoperative Care

Documentation of postoperative care is very important. A simple rule to follow is: “if it isn't written down, it didn't happen.” The USDA requires that health care records be maintained in a manner consistent with prevailing professional veterinary practice standards.
For animals larger than rodents, individual health care records are usually maintained, with records of daily observations and treatments during the postoperative care period.

For smaller animals such as rodents, group records instead of individual records are usually kept. The veterinary staff or the research staff may maintain the records, but the records should always be accessible to the veterinary staff should complications arise. The records should be maintained at least a year after the death of the animal to meet USDA policy.

b. Postoperative Recovery Period
In the absence of complications, the postoperative period traditionally ends between 10 and 14 days after surgery, when skin sutures are often removed. After that, routine daily monitoring can be resumed, and routine entries in the health records discontinued.

The USDA does not allow changes in animal ownership during the postoperative recovery period, and does not allow movement of the animal between facilities during recovery from anesthesia unless the IACUC approves it. These prohibitions are meant to help ensure continuity of care during the postoperative period. Appropriate health records must be maintained regardless of the animal's location.

18. Fasting
Animals are often fasted prior to surgery so that the risk of aspiration pneumonia is minimized. Aspiration pneumonia can occur if an animal vomits, then breathes ("aspirates") the vomit into the lungs. For this reason, fasting is often recommended. **Rodents and rabbits, however, are unable to vomit because of their gastrointestinal anatomy, and thus they should not be fasted before surgery unless there are other medical or scientific reasons for doing so.**

19. Common Non-Surgical Procedures
These procedural descriptions below have been approved by the GSU IACUC for incorporation into an investigator's protocol. Investigators conducting one or more of these procedures can indicate adherence to the procedural description on their IACUC protocol or request a deviation from the procedural description for consideration by the IACUC as necessary.

a. Blood Collections
   **Auxiliary Cut down:**
   This is a terminal procedure with the objective to collect the maximum amount of blood possible.
   1. The animal will be anesthetized.
   2. With the animal in dorsal recumbency (lying on its back), the axillary (armpit) area will be prepped with alcohol swab.
   3. A cut will be made in the axillary region with scissors or a scalpel blade to expose the subclavian artery and vein which are deep in the armpit.
   4. The subclavian artery and vein will be cut with the scissors or a scalpel blade.
5. The blood sample will be collected with the syringe (no needle) as the blood pools in the axillary region.

6. After the procedure, a bilateral thoracotomy will be performed to assure the animal has been euthanized.

Cardiac Puncture:
This is a terminal procedure with the objective to collect the maximum amount of blood possible.

1. The animal will be anesthetized.
2. With the animal in dorsal recumbency (lying on its back), the area will be prepped with an alcohol swab.
3. A needle will be inserted at base of sternum at a 20-30 degree angle just lateral of the midline on the animal's left side. The thumb and index finger will be used to feel the heartbeat to assist in directing the needle.
4. The syringe will be aspirated slowly while advancing.
5. Alternatively, the procedure may be performed with the animal on its right side. In this case the needle will be inserted just behind the forelimb where the heartbeat is easily felt.
6. After the procedure, a bilateral thoracotomy will be performed to assure the animal has been euthanized.

Facial Vein (Mouse Only):

1. The animal will be restrained via hand restraint. The skin should be taut over the mandible.
2. A 25 gauge needle or appropriate size lancet will be used to puncture the facial vein which is located slightly behind the mandible but in front of the ear canal. A swift lancing motion is used to puncture the vessel. Only the tip of the needle should enter the vessel to a shallow depth of 1-2 mm. Blood will flow immediately.
3. The blood will be collected with a pipette or other collection tube.
4. Upon completion, good hemostasis will be ensured via applying gentle pressure to the blood collection site with a gauze sponge.
5. The animal may be returned to their home cage once they have fully recovered from the anesthesia.
6. Regarding collection amount, no more than 1% of the animal's body weight will be collected in a two week interval. For example, if the animal weight 25 grams, no more than 0.25 ml of blood will be collected in a two week interval.

Retro-Orbital:

1. The animal will be anesthetized.
2. After the animal is anesthetized, a drop of Proparacaine Hydrochloride (local anesthetic) will be placed in the eye from which the sample is to be collected unless the procedure is conducted as a terminal (non-survival) procedure. The Proparacaine Hydrochloride takes effect in about 30 seconds and lasts for about 15 minutes.
3. A hematocrit tube will be placed at the medial canthus of the eye and inserted behind the eye.
4. The tube will be rotated gently against the back of orbit until blood flows. Please note that this is a finesse procedure and does not require force.
5. Upon completion, good hemostasis will be ensured by holding eyelids closed.
6. Regarding collection amount, no more than 1% of the animal's body weight will be collected in a two week interval. For example, if the animal weight 25 grams, no more than 0.25 ml of blood will be collected in a two week interval.

*Animals that receive retro-orbital sinus blood collections should be monitored for adverse signs such as swelling around the eye, protrusion of the eye, sunken eye, squinting eye, or hemorrhage around the eye, the day of and the day following blood collection. If any of these signs are noticed the DAR veterinary staff should be consulted.

Saphenous (Recommended Blood Collection Procedure for Survival Surgeries)
1. The animal will be restrained via hand restraint or restraint device.
2. Hair will be clipped from lateral aspect of lower leg. A straight razor may also be used to clip the fur from the leg.
3. The saphenous vein will be lightly constricted above the knee joint and petroleum jelly applied over the shaved area of the leg as this will facilitate blood flow and help prevent clotting during collection.
4. The vein will be punctured with a needle (typically 22 or 23 gauge). Blood will be collected with a hematocrit tube or other collection tube.
5. Upon completion, good hemostasis will be insured by applying gentle pressure to the collection site.
6. Regarding collection amount, no more than 1% of the animal's body weight will be collected in a two week interval. For example, if the animal weighs 25 grams, no more than 0.25 ml of blood will be collected in a two week interval.

Tail Nick:
1. The tail veins will be dilated by one of three methods: placing a heat lamp over the cage of animals for no more than 5 minutes; placing the tail in warm water (temperature not to exceed 47 degrees Celsius) for a couple of minutes; rubbing the tail for several seconds with a gauze pad soaked in very warm, but not hot, water.
2. The animal will be restrained in an appropriate sized restraint device with the tail extended. Tail will be cleaned with alcohol.
3. The tail veins are located on either side of the tail. Starting 2-3 cm from the tail tip, the vein will be nicked with a 23 – 25 gauge needle or sterile scalpel blade.
4. Blood may be collected with micro capillary tubes, a micropipetette or various microtainer collection tubes. The tail can be gently stroked from the base of the tail toward the tail to encourage blood flow.

5. Upon completion, good hemostasis will be insured by applying gentle pressure to the collection site.

6. Subsequent blood samplings may be obtained in animals by removing the scab. If blood is not obtained upon removing the scab, one can proceed to collect an additional blood sample by performing a tail nick on the opposite of the tail.

7. Regarding collection amount, no more than 1% of the animal's body weight will be collected in a two week interval. For example, if the animal weighs 25 grams, no more than 0.25 ml of blood will be collected in a two week interval.

**Tail Snip:**

Pain perception of tail clamping in rats does not start to develop until 12 to 14 days of age, so performing tail biopsy earlier in rodents may cause less pain. When performed properly in adult mice it causes only minimal or transient pain and distress, and induces no more “physiological impact” (change in heart rate, body temperature, or activity level) than restraint of the animal for the procedure.

For mice and rats 12 days of age and younger: Tail biopsies should preferably be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, bleeding is minimal, and anesthesia is not required.

For mice and rats 13-21 days of age: Based on the physiological impact and rodent pain ontogeny studies, investigators are required to utilize an analgesic regimen such as immersion of the tail in ice cold ethanol for 10 seconds, topical application of ethyl chloride spray, by injection of an analgesic (e.g. carprofen, buprenorphine, etc.) at least 20 minutes prior to the conduct of the procedure, etc.

For mice and rats greater than 21 days of age: The use of an analgesic or general anesthetic is required prior to collection of tissue.

For mice and rats greater than 35 days of age: The use of a general anesthetic is required.

To conduct the procedure, manually restrain the mouse or rat between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc.). With sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should be enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. If small amounts of DNA are required, investigators should consider taking only 2 mm of
tail. If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also disinfect the work surface on which the tail is placed between animals.

The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. If needed, apply digital pressure, silver nitrate, or some other means of hemostasis. Each animal will typically only be genotyped once. However, if a second tail snip is needed (e.g. due to a technical error with the PCR, etc.) then only one additional tail snip is permissible and the total amount of tail removed will not exceed 5 mm (first and second snip combined).

**Wing Veins (Birds)**

1. The bird will be captured by hand from one of the large flight aviaries (this is done by switching off the lights and inducing perching behavior then gently capturing the bird with both hands).
2. The bird will be gently restrained with one hand while the other hand fully extends one wing.
3. A second person will then locate the alar vein on the inside of the wing (there are few, if any, feathers here and plucking them is not necessary) and will wipe the area with 70% alcohol.
4. The alar vein will be gently punctured with a sterile hypodermic needle. The blood will be collected as it drips from the vein into an appropriate collection devise into 2 or 3 heparinized capillary tubes (150-210 microliters total).
5. Upon completion, good hemostasis will be insured by applying gentle pressure to the collection site.
6. Regarding collection amount, no more than 1% of the animal's body weight will be collected in a two week interval. For example, if the animal weight 100 grams, no more than 1 gram of blood (equivalent to 1 ml of blood) will be collected in a two week interval.

**b. Injections**

**Retro-Orbital Sinus:**

1. The animal will be anesthetized.
2. After the animal is anesthetized, a drop of Proparacaine Hydrochloride (local anesthetic) will be placed in the eye which is to be used for the injection. The Proparacaine Hydrochloride takes effect in about 30 seconds and lasts for about 15 minutes. Excess proparacaine is carefully dabbed away (e.g. with a gauze pad).
3. The animal will be positioned on its side. The skin above and below the eye will be pulled back (e.g. with the thumb and index finger) so the eye will slightly protrude.
4. A 27 gauge (or smaller diameter) 1/2 inch long needle connected to a 1 cc syringe will be placed at the medial or lateral canthus of the eye at a 30 degree angle and will be gently inserted a few mm behind the eye (into the retro orbital space). NOTE: the needle is inserted bevel down.
5. The agent will be slowly inject into the retrobulbar sinus (over a period of a few seconds). No more than 150 microliters will be injected.
6. The needle will be gently and slowly removed (over a period of a few seconds).
7. If an injectable anesthetic was used, sterile eye ointment will be instilled when finished. If isoflurane anesthetic was used then sterile eye ointment is not required because the animals will recover from anesthesia quickly.
8. Upon completion, good hemostasis will be ensured by holding eyelids closed for a few seconds.
9. No more than two retro-orbital injections, in total, will be performed on a given mouse and opposite eyes will be utilized with a minimum interval between procedures of 7 days.

**Intradermal (ID):**
*The injection volume will not exceed 0.1 ml.
1. The animal will be anesthetized.
2. The hair on the back will be clipped and prepped with an alcohol swab.
3. A needle will be inserted between layers of skin on the back at a 30-degree angle.
4. Syringe will be aspirated to insure proper placement. Any sign of blood or other fluid indicates improper placement - reposition.
5. Article will be administered in a steady, fluid motion to avoid tissue trauma. Successful injection results in a small circular skin welt.

**Intramuscular (IM):**
*The injection volume will not exceed the following parameters: MOUSE and SIBERIAN HAMSTER ≤0.05mL using smaller than 23G needle; RAT ≤0.3 mL using smaller than 21G needle; SYRIAN HAMSTER ≤0.1 mL using smaller than 21G needle
1. The animal will be restrained.
2. Area will be cleaned with alcohol.
3. Needle will be inserted into hind leg muscle (either in front of the thigh bone or, if behind it, direct the needle towards the back of the leg).
4. Syringe will be aspirated to insure proper placement. Any sign of blood in the syringe indicates improper placement - reposition.
5. Article will be administered in a steady, fluid motion. DO NOT administer rapidly because this may cause tissue trauma.

**Intraperitoneal (IP):**
*The injection volume will not exceed the following parameters: MOUSE and SIBERIAN HAMSTER 2-3 mL using smaller than 21 G needle; RAT 5-10 mL using smaller than 21 G needle; SYRIAN HAMSTER 3-4 mL using smaller than 21 G needle.
1. The animal will be restrained, tilting the body at a 45-degree angle with the head down. This will position the intestines cranial to the injection site.

2. Area will be cleaned with alcohol.

3. A needle will be inserted into the animal's right lower quadrant of the abdomen at a 30-degree angle.

4. Syringe will be aspirated to insure proper placement. Any sign of blood or other fluid indicates improper placement. To prevent inducing peritonitis, remove syringe, discard, and use new syringe, needle, and article in the event that fluids other than blood are aspirated.

5. Article will be administered in a steady, fluid motion.

**Lateral Tail Veins:**

Note: The secret of successful injection of the tail vein is to dilate the veins. This has been accomplished in various ways such as the following: placing the tail in warm water (47 degrees Celsius for about 1 minute (do not exceed 47 C as this can result in thermal injury to the tail); placing the animal in an incubator (37° C) for 10 to 15 minutes; carefully using a heat lamp on the tail for 1-2 minutes; or wrapping the tail in an electric heating pad that is warm (not hot) to the touch. In addition one can place a tourniquet around the base of the tail to facilitate visualization of the vein (a rubber band and mosquito hemostat are suitable for this purpose).

*The injection volume will not exceed ~1% of the animal's body weight in mL (i.e., 0.25 mL for a 25 g mouse).

1. The animal will be restrained.

2. The tail will be warmed as mentioned above to facilitate dilation.

3. Needle placement will be no closer to the body than half the length of the tail.

4. With tail under tension, the needle will be inserted into skin approximately parallel with the vein.

5. Proper placement will be insured by inserting needle at least 3 mm into lumen of vein.

6. Article will be administered in a steady, fluid motion to avoid rupture of vessel.

7. Upon completion, good hemostasis will be insured by applying gentle pressure before returning to cage.

**Subcutaneous:**

*The injection volume will not exceed the following parameters: MOUSE and SIBERIAN HAMSTER 2-3 mL using smaller than 20 G needle; RAT 5-10 mL using smaller than 20 G needle; SYRIAN HAMSTER 3-4 mL using smaller than 20 G needle.

1. The animal will be restrained.

2. Area will be cleaned with alcohol.

3. Needle will be inserted at base of skin fold between thumb and opposing finger.
4. Syringe will be aspirated to insure proper placement. Any sign of blood indicates improper placement; also, a lack of negative pressure in the syringe indicates the needle has punctured out through the opposite side of the skin - reposition.

5. Article will be administered in a steady, fluid motion.

c. Other Procedures:

   Oral Gavage:

   *Maximum administration volume = 10 ml/kg*

   1. The distance from the tip of nose to the last rib will be measured as this constitutes the length gavage tube will be inserted be inserted.

   2. The animal will be restrained via hand restraint

   4. The tip of gavage tube will be placed in the rear of the animal’s mouth to induce swallowing.

   5. The tip will be slid down back of mouth, moving tip forward in one fluid motion.

   6. Take your time, any resistance felt indicates improper placement. Tube should slide down into esophagus easily. A violent reaction (coughing, gasping) usually follows accidental introduction of the tube into the larynx or trachea.

   7. Using the gavage tube to gently extend the neck facilitates introduction into the stomach.

   8. Once the needle is properly placed, the article will be administered in a steady, fluid motion.

20. Medical Record Keeping

Animal medical, surgical, and research records are a key element of a program of adequate veterinary care as it relates to the animal care and use program. The animal medical, surgical, and research record keeping system delineated herein was developed in congruence with the guidance provided by the American College of Laboratory Animal Medicine (2007. Medical Records for Animals Used in Research, Teaching, and Testing: Public Statement from the American College of Laboratory Animal Medicine; ILAR Journal 48(1):37-41).

Medical Records: Regarding the development of spontaneous disease (e.g. disease other than experimentally induced disease such as fight wounds, spontaneous tumors, dental problems, etc.), the relevant animal observations, treatments, and disease outcome (be it disease resolution or euthanasia) are recorded in the electronic Laboratory Animal Care Record (LACR) which includes the medical record and treatment sheet. The LACR medical record is located on a password protected website “DropBox.” Should an animal be found to be in need of immediate medical attention (whether or not the disease condition was spontaneous or experimentally produced) and, if the DAR staff is unable to reach a member of the research laboratory, the DAR veterinarian is authorized to act on behalf of the animal (treatment or euthanasia). Otherwise, the DAR veterinary staff would make decisions regarding treatment or euthanasia in consultation with the research lab.
A hardcopy of the LACR will be associated with each IACUC protocol represented in the animal housing room. The hardcopy is available for the research staff to record any relevant animal observations, treatments, etc. This record is maintained in the 3-ring binder located in the respective animal room. Blank copies of the LACRs are found in the 3-ring binder and are also found online (http://ursa.research.gsu.edu/ursa/compliance/animal-resources-program/health-record-system). Should the researcher observe an animal health issue, they can make a notation on the LACR, place a red sticker on the appropriate cage card (red stickers are located in the 3-ring binder located in the respective animal room), and contact the DAR Animal Healthcare Technicians (office: 404-413-3594; mobile: 404-709-9910 or 404-908-4933; e-mail: cbillinglsey2@gsu.edu or jscott72@gsu.edu) or the veterinarians (Mike Hart: office: 404-413-3553; mobile: 404-391-7366; e-mail: mhart@gsu.edu or Amelia Wilkes: office: 404-413-3636; mobile: 678-923-0970; e-mail: ajones234@gsu.edu). Should the laboratory animal technician observe an animal health issue, they place a red sticker on the appropriate cage card, and send an email to the GSU animalhealthreq@gmail.com describing the observation. In the event of an emergency the laboratory animal technician staff or research should contact the above individuals using their mobile phones.

Surgical Records: Notations related to the conduct of surgical procedures (whether survival or non-survival) must be recorded on the “Animal Surgical Record.” This record is maintained in the 3-ring binder located in the respective animal room. Blank copies of the Animal Surgical Record are found in the 3-ring binder and are also found online (http://ursa.research.gsu.edu/ursa/compliance/animal-resources-program/health-record-system). This record may reflect the surgery of a single or multiple animals on a given day.

Research Records: Notations related to disease that is experimentally induced or experimental procedures that are conducted on animals do not necessarily need to be maintained in the medical record. Rather, it is typically appropriate for this information to be retained within a research record so long as this information is readily available for review by the veterinary staff, as well as for appropriate internal (e.g., IACUC) or external (e.g., USDA, PHS, AAALAC) oversight entities. If research data pertaining to experimentally induced disease or animal procedures conducted cannot be readily retrieved from a researcher’s notebook or computerized database, then this research data should be included within the medical record (Laboratory Animal Care Record) located in the animal housing room. An example of research data which must be documented includes the following:

1. Animal or group identification and the date of the procedure
2. Substances administered, including dose and route
3. Blood collection, euthanasia
4. Monitoring for animal pain and distress and humane endpoints consistent with the parameters approved in section 4.3 of the IACUC protocol
5. All entries in the record should be dated and indicate the originator of the entry (e.g., initials, signature/electronic signature) and be legible to someone other than the writer.
6. Regarding the administration of infectious agents in animals, DAR will provide cage labels for the researcher to note the name of the infectious agent and the date it was administered to the animal.

7. Regarding the administration of medicated/special feed or medicated water, DAR will provide cage labels which contain a place to record the substance which has been added to the water or which clarifies the medicated/special feed.

8. Regarding breeding rodents, DAR will provide cage cards which contain places to record relevant breeding information.

9. Regarding the administration of tumor cell lines, DAR will provide cage labels for the researcher to note the date of injection and location of injection (on the animals).

10. Regarding animals receiving chemical hazards, all standard operating procedures related to the use of such hazards must be followed. In addition, DAR will provide cage labels which contain a place to record the name of the chemical hazard which has been administered to the animal as well as the date of administration.

11. Regarding animals undergoing total body irradiation, DAR will provide cage labels for the researcher to note the date of the total body irradiation.

12. Regarding animals undergoing food or water restriction, DAR will provide cage labels for the researcher to note the date and time the restriction commenced and will terminate.

21. **Use of Expired Drugs or Materials**

Per the PHS Policy on the Humane Care and Use of Laboratory Animals, the use of expired pharmaceuticals, biologics, and supplies is not consistent with acceptable veterinary practice or adequate veterinary care. Euthanasia, anesthesia and analgesia agents should not be used beyond their expiration date, even if a procedure is terminal. As it relates to other drugs or materials, the GSU IACUC does not oppose the use of expired medical materials for terminal procedures if their use does not adversely affect the animal’s well-being or compromise the validity of the scientific study.

All expired medical materials must either be disposed of or segregated from non-expired medical materials and conspicuously labeled as expired while either awaiting disposal or while awaiting use in a terminal study. Each research laboratory is expected to monitor its inventory of medical materials used on animals (e.g. drugs, sutures, fluids, etc.) to ensure adherence to this policy. Research laboratories have access to the Chematix Chemical Management Software system should they desire to inventory all of their medical materials containing expiration dates utilizing this system. If utilized for this purpose, this system will, in turn, notify the principal investigator and designated laboratory supervisor via e-mail regarding pending expirations.

22. **Use of Non-Pharmaceutical-Grade Substances**

The use of pharmaceutical-grade substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals and ensures the health and welfare of the animals. Federal regulation requires that investigators use pharmaceutical-grade substances in live animals being used in research and teaching.
whenever they are available, even in acute procedures. Pharmaceutical-grade substances are ones which are approved by the U.S. Food and Drug Administration or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary or the British Pharmacopeia.

It is understood that the administration of non-pharmaceutical-grade substances may be necessary in order to meet the scientific goals of a project or when a veterinary or human pharmaceutical-grade product is not available. For instance, in studies seeking to test novel compounds, no pharmaceutical-grade compound would be available. In addition, it is recognized that, in some cases, the available human or veterinary drug is not concentrated enough to meet experimental requirements. The use of non-pharmaceutical-grade substances should be based on 1) scientific necessity, 2) non-availability of an acceptable veterinary or human pharmaceutical-grade compound, and 3) specific review and approval by the IACUC. Cost savings is not a justification for using non-pharmaceutical-grade compounds.

**Preparation of Non-Pharmaceutical Grade Substances**

- Filtering: filter through a 0.2μm membrane filter
- pH Testing: the pH should be between 6.8-7.2.
- Osmolarity Testing: the final solution should be isotonic, with an osmolarity around 300 mOsm.
- A sterile diluent should be used (e.g. sterile physiological saline)
- The substance should be stored in a sterile injection vial
- The injection vial must indicate the name of the substance, the concentration, and the date of expiration. Regarding date of expiration, please note the following: Single compounds: The date listed on the original bottle or box; Mixtures/Compounds: The earliest date listed on any agent which is mixed or compounded; Experimental compounds: Compounds without an expiration date will be discarded based on performance evaluation of the agent(s)
- Multiple-dose injectable vials should not be used if they contain particulate matter, precipitates, turbidity, or discoloration.

**Use of Hazardous and Toxic Agents**

The Georgia State University Biosafety Committee, the Radiation Safety Committee, and appropriate safety officers are charged with evaluating safe practices for using hazardous agents in animals.

Guidelines for performing infectious disease work with animals are found primarily in the Centers for Disease Control and Prevention (CDC)/NIH publication entitled "Biosafety in Microbiological and Biomedical Laboratories," or "BMBL." The BMBL has guidelines for working with a wide variety of infectious agents in both research laboratories and the animal facility.

Any Hazardous or toxic agents or radiological material use require an approved Institutional Biosafety Committee Protocol and/or Radiation Protection Committee Protocol. The IACUC protocol will not be approved until all approvals from other committees are provided to the IACUC. Please submit your protocols to the appropriate committees as soon as possible to avoid any delays in the approval of your IACUC protocol.
If your animal work requires the use of hazardous or toxic agents, there are many important considerations. Such agents can be categorized in the following ways:

- Infectious diseases.
- Toxic chemicals, including carcinogens, mutagens, biological toxins, and organic chemicals.
- Radioactive substances.
- Recombinant DNA.

Some points to consider when using such agents in animals:

- The **risk of accidental human infection or exposure is usually reduced if animals are anesthetized or sedated before they are injected with agents using a hypodermic needle.** Anesthetized animals will not struggle unpredictably and this helps prevent accidental redirection of the needle towards the personnel handling the animals.
- If using an infectious agent, **an antibiogram or other appropriate therapeutic panel should be developed on infectious strains** before they are used in animals. If an accidental human exposure occurs, physicians will know immediately which antibiotic or other therapeutic agent to use to best treat the infection. Consult with your occupational health staff in advance of experiments.
- If using a toxic agent, **know in advance what antidote or action to take if accidental exposure should occur through an injection, spill, or break in the skin.** Have any necessary antidotes, decontamination kits, or spill kits readily available. Consult with the appropriate safety officer in advance of experiments.
- When administering hazardous agents to animals, it is best for personnel to **work in pairs.** If one person becomes contaminated, the second person can help decontaminate the person and the area quickly.

**a. Biosafety and Animal Biosafety Levels**

There are four levels of containment procedures for infectious agents recognized in the BMBL. The levels are designated **Biosafety Levels 1, 2, 3, and 4.** The containment and handling safeguards become more stringent as the biosafety level (or “BSL”) number increases. For each of the four biosafety levels, there are corresponding **Animal Biosafety Levels 1, 2, 3, and 4** (or "ABSL") that provide guidelines for housing and manipulating animals infected with agents that require that level.

Before beginning any animal studies involving infectious agents, both research staff and personnel in the animal facility must understand how to safely conduct the study in the animal facility. **Standard Operating Procedures (SOPs) must be written and approved by the Biosafety Committee before any infectious work begins,** and you may be required to provide a detailed SOP prior to the IACUC review. An SOP should describe how animals will be handled and housed in the animal facility after they are infected. It is very important that the veterinarian, biosafety officer, biosafety committee, and animal facility manager be involved in the planning as needed to minimize the risk of exposing humans or other animals to the agent.
b. Select Agents
The Department of Health and Human Services enforces "Additional Requirements for Facilities Transferring or Receiving Select Agents" for certain infectious agents and biological toxins. These "select agents" are given additional regulatory oversight because of their potential use in biological warfare. The list of select agents includes infectious agents like hemorrhagic fever viruses, and plague, brucellosis, and anthrax bacilli. It also includes a number of biological toxins such as aflatoxin and botulinum toxins. An investigator must register with the Centers for Disease Control and Prevention and obtain approval before beginning any work with agents on the "select agent" list.

c. Toxic Chemicals and Radioisotopes
As with infectious disease studies, the use of toxic chemicals or radioisotopes in animals requires careful coordination between many people, including:

- The research staff.
- The veterinarian and animal facility supervisor.
- The appropriate institutional units or committees (Office of Research Compliance and Safety, Radiation Safety Committee, Chemical Safety Officers) responsible for use of hazardous agents.

The IACUC will ensure that all research and animal facility personnel are trained to properly minimize the risk of accidental human or animal exposure. SOPs describing containment and handling procedures should be written and approved by appropriate committees before any animal work begins. It is critically important to also train the animal caretakers who will clean, feed, and water the animals. If highly specialized training is required to handle animals safely, then the research staff might have to assume husbandry duties for infected animals.

d. Recombinant DNA
There are additional guidelines to consider if your work in animals includes: http://oba.od.nih.gov/oba/rac/guidelines/nih_guidelines.htm

- The inoculation of infectious agents or cells with recombinant DNA into animals, or
- The use of recombinant molecules in an animal’s genome.

The purpose of the NIH Guidelines for Recombinant DNA and Gene Transfer are to specify practices for constructing and handling recombinant deoxyribonucleic acid (DNA) molecules and organisms and viruses containing recombinant DNA molecules.

Although the recombinant DNA inserted into many transgenic mice may not be covered, it is wise to check with the Biosafety Committee before you produce transgenic mice to determine what committee approvals will be necessary.

Experiments involving recombinant DNA must be reviewed by the IBC. In some cases an NIH committee called the "Recombinant DNA Advisory Committee" must also approve the experiments. Experiments involving very high risk, such as introducing novel antibiotic resistance into human pathogens, may also
require NIH approval before initiation. Guidance can be obtained from the Office of Biotechnology Activities (OBA) at the NIH.

e. Risk Categories

The NIH Guidelines for Recombinant DNA and Gene Transfer document describes different levels of agent containment practices very similar to the levels described in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories manual for infectious agents. [http://www.cdc.gov/biosafety/publications/bmbl5/](http://www.cdc.gov/biosafety/publications/bmbl5/)

If recombinant nucleic acid is introduced into infectious agents, the level of laboratory and animal facility containment required is primarily based upon the "Risk Category" assigned to the agent involved. The Risk Categories are based upon disease potential in healthy humans and availability of therapy. For each Risk Category, there is a corresponding set of animal biosafety guidelines that must be used, as follows:

- **Risk Category 1** (RC-1). Agents not associated with disease in healthy adult humans. Generally, if an RC-1 agent is used in animals, "Biosafety Level 1-N" (BSL1-N) animal containment measures are used.
- **Risk Category 2** (RC-2). Agents associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. Generally, if an RC-2 agent is used in animals; BSL2-N animal containment measures are used.
- **Risk Category 3** (RC-3). Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). Generally, if an RC-3 agent is used in animals; BSL3-N animal containment measures are used.
- **Risk Category 4** (RC-4). Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). Generally, if an RC-4 agent is used in animals; BSL4-N animal containment measures are used.

The containment practices recommended for each of the animal biosafety levels (BSL1-N through BSL4-N) are found in **Appendix Q** of the NIH Guidelines for Recombinant DNA and Gene Transfer document. [http://oba.od.nih.gov/rdna/rdna.html](http://oba.od.nih.gov/rdna/rdna.html)

f. Explosive Agents in the Animal Facility

In the Georgia State University animal facilities, the use of ether to anesthetize or euthanize animals, and the use of other explosive agents are prohibited unless there are compelling scientific reasons for not using non-explosive alternatives. Check with the Office of Research Compliance and Safety and the IACUC before using any explosive agents in the animal facility.

If you are approved to use explosive agents such as ether to euthanize animals, **DO NOT** put the bagged carcasses in a refrigerator or freezer UNLESS you are absolutely certain that all of the agents have evaporated from the carcasses, and then only if the refrigerator or freezer is certified as explosion-proof. Sparks
produced by non-explosion-proof refrigerators and freezers can ignite the fumes given off by the carcasses and cause a tremendous, deadly explosion.

In addition, you must be aware that ether stored in metal cans will form highly unstable peroxides around the can lid over time. These peroxides can become so unstable that they can detonate if the can is jarred. If you have old cans of ether in the chemical storage vault in your laboratory area, consult with the Office of Research Compliance and Safety to make sure that they do not represent an explosion hazard.

24. Monitoring of Biological Materials

The injection of transplantable tumors, hybridomas, cultured cell lines, or other biological materials into rodents can pose a health risk to animals and personnel. These biological materials have been a source of mouse hepatitis virus, mouse pox, and other significant disease agents at research facilities. Moreover, rodent pathogens can be carried and propagated by non-rodent (e.g. human) cell lines when these cell lines have been propagated in rodents or rodent biological materials. Similarly, there is a concern with the potential for contamination of biological materials of human origin with human pathogens as posing a risk to personnel handling the specimens or handling immunocompromised mice harboring such xenografts.

Biological materials should be evaluated for rodent pathogenic microorganisms by polymerase chain reaction (PCR) or mouse antibody production (MAP) tests. The major disadvantage of MAP testing is the 6 to 8 weeks required to obtain results. The IDEXX Bioresearch lab offers a PCR-based alternative to MAP testing, the Infectious Microbe PCR Amplification Test or IMPACT, which is a panel of PCR assays that detects murine pathogens. Regarding human cells, IDEXX BioResearch has developed and validated a selection of PCR assays (h-IMPACT) for the testing of biological samples for the presence of selected human pathogens if such testing has not already been conducted. Typically, IMPACT testing requires 2 vials of each sample with a minimum of 1 x 10^7 cells/vial and a turnaround time of 7-10 days.

If your protocol involves the injection of transplantable tumors, hybridomas, cultured cell lines, or other biological materials into rodents, please provide a DAR veterinarian the name of the cell line(s), source, test, and results of tests performed to evaluate the presence of rodent pathogenic microorganisms (and/or human pathogenic microorganisms, if applicable). If the cells are not of rodent origin and have not been tested for the presence of rodent pathogens, please confirm that the materials (cells) to be used have not been propagated in rodents or rodent biological materials. Alternatively, please contact a DAR veterinarian to make arrangements to have biological specimens tested before use by completing the Cell Line Use Request Form (http://ursa.research.gsu.edu/files/2013/04/Cell_Line_Use_Request_Form.doc). Approval for the use of biological materials in animals housed at GSU will only be given after a DAR veterinarian has assessed the test results to determine their adequacy.

I. Husbandry

1. Animal Acclimation after Arrival

Newly received animals must be provided a period for physiologic, psychologic, and nutritional stabilization before their use, consistent with the recommendations in the
Guide for the Care and Use of Laboratory Animals. All animals utilized on IACUC-approved protocols must be provided an appropriate stabilization period after arrival at GSU before they may be used on one’s protocol. Animals must be allowed to acclimate to their new environment for a minimum of 3 days prior to their use in experimental procedures. Animals to be used for acute tissue harvest may be used immediately after arrival provided that the investigator is aware that data may be affected by the stress and decreased food and water intake associated with shipping.

2. Animal Enrichment

Besides the basic needs of food and water, animals require some form of enrichment to maintain physiological and psychological well-being. The DAR has implemented the following program to provide enrichment to all animals unless otherwise directed by Principal Investigators (in the cases where enrichment would provide a confounding variable in their research). The plan is broken down by species.

Syrian Hamsters (Mesocricetus auratus)

Syrian hamsters are provided nestlets to make a nest. Syrian hamsters are group housed when possible which provides social enrichment. Hamsters may also receive shelters.

Siberian Hamsters (Phodopus sungorus)

Siberian hamsters are provided nestlets to make a nest. Siberian hamsters are group housed when possible which provides social enrichment. Siberian hamsters may receive running wheels and split-level caging to simulate a burrowing environment. Hamsters may also receive shelters.

Mice (Mus musculus)

Mice are provided nestlets to make a nest. Mice are group housed when possible which provides social enrichment. Mice may also receive gumma-bones and/or shelters. Nude mice receive nesting sheets in lieu of nestlets.

Rats (Rattus norvegicus)

Rats may receive Nylabones® for chewing and/or shelters. Breeding rats receive shredded paper bedding with which to build a net. Rats are group housed when possible which provides social enrichment.

Rabbits (Oryctolagus cuniculus)

Rabbits are given both nutritional and physical enrichment. Rabbits receive toys (dumbbells, jingle balls) ad lib. Rabbits receive timothy hay cubes daily (when not on restricted/transitional diet), fresh vegetables at least weekly (typically carrots and broccoli), and a Bunny Block® (Bioserv) once a month. Some rabbits are pair/group housed depending on size and compatibility. Additionally, a “playpen” area is in place to allow individual and pair/group housed rabbits to forage and play in a larger area.

Ferrets (Mustela putorius furo)

Ferrets are given shredded paper bedding and ferret balls to sleep/play in and other toys as appropriate. Ferrets are group housed when possible which provides social enrichment.

Cotton Rats (Sigmodon hispidus)
Cotton rats may receive Nylabones® for chewing and/or shelters. Cotton rats are group housed when possible which provides social enrichment.

**Zebra Finches (Taeniopygia guttata)**
Zebra Finches are mostly group or pair housed. They are provided millet once a week. Their cages are stocked with multiple perches, ladders, cuttlebones, and bird toys. Most finches are maintained in spacious flight cages.

**Lizards (Anolis carolinensis)**
Lizards have perches, plants, and a water misting system. They also receive live crickets for food/enrichment. Lizards are group housed when possible which provides social enrichment.

**African Clawed Frogs (Xenopus laevis)**
African clawed frogs receive a PVC pipe for shelter. Frogs are group housed when possible which provides social enrichment.

**Tree Frogs (Hyla cinerea)**
Tree frogs have perches, plants, and a water misting system. They also receive live crickets for food/enrichment. Frogs are group housed when possible which provides social enrichment.

**Fish (Various Species)**
Fish may have shelters (PVC tubes). Fish are group housed when possible which provides social enrichment.

**Chimpanzees (Pan troglodytes)**
All chimpanzees are socially housed with con specifics. They have access to both outdoor and indoor primary enclosures. Cage props include but are not limited to: ropes, fire hoses, swinging bars, and hammocks. They are challenged to solve problems, obey requests, and perform specific tasks. They have control over many aspects of their environment (e.g. they can ask for specific foods, to go to specific places, to do specific things such as chase, tickle, watch a movie, etc.). Multiple forms of stimulus object enrichment are utilized (e.g. shiny magazines or advertisements with staples removed; cardboard shipping boxes of various sizes; towels given at night and naptime for nest building and then retrieved for cleaning; ice blocks with frozen treats; PVC tubes filled with peanut butter, honey, popcorn, seeds, nuts, raisins, or other treats, etc.). Additionally, enrichment foods such as various fruits and vegetables are provided as a component of the daily ration.

**Rhesus Monkeys (Macaca mulatta)**
Each rhesus monkey has access to its home cage (a Britz “play cage”) which is connected to a “tunnel system.” Thus, a given monkey can choose to leave its home cage and enter a section of the tunnel system as an expansion of its home cage. In turn, each tunnel system is connected to an outdoor turnout. Doorways in the tunnel system allow one to control physical access of compatible monkeys to one another and these doorways will be opened once a day for a couple of hours to allow compatible monkeys access to one another to include their outdoor turnout. All rhesus macaques have continuous visual and vocal access to one another. They are afforded 16-24 hour/day access to a computerized
testing system. Each monkey works ad libitum. Manipulation of a joystick in response to various task demands results in delivery of nutritive rewards. Many days, the monkeys can choose not only when to work, but also on what task to work; that is, a menu of task options is made available to the animals. Each individual cage is equipped with a perch located approximately 36” above the cage floor. The outdoor turnouts also have perches and other climbing structures. Each monkey is provided access to at least one manipulandum, in addition to the computerized test system. Manipulanda includes such items as toys; swings; mirrors; grooming boards coated with pellet dust or crumbles; PVC tubes filled with peanut butter, honey, popcorn, seeds, nuts, raisins, or other treats to the animals; raisin boards; and puzzle feeders. Additionally, enrichment foods such as various fruits and vegetables are provided as a component of the daily ration.

**Capuchin Monkeys (Cebus capucinus)**

All capuchins are socially housed with con-specifics. They have access to both outdoor and indoor primary enclosures at all times except during research testing, inclement weather, cleaning or cage repair and modification. Cage props include but are not limited to platforms, swings, ropes and a water shower. They are challenged to solve problems, obey requests, and perform specific tasks. Monkeys are routinely afforded access to a computerized testing system. Each monkey works ad libitum. Manipulation of a joystick in response to various task demands results in delivery of nutritive rewards. Many days, the monkeys can choose not only when to work, but also on what task to work; that is, a menu of task options is made available to the animals. Monkeys also receive manipulanda including items such as toys; swings; mirrors; grooming boards coated with pellet dust or crumbles; PVC tubes filled with peanut butter, honey, popcorn, seeds, nuts, raisins, or other treats to the animals; raisin boards; and puzzle feeders. Additionally, enrichment foods such as various fruits and vegetables are provided as a component of the daily ration.

### 3. Animal Transportation

When an IACUC approved protocol requires animal transportation to an investigator’s lab or from one GSU animal facility to another GSU animal facility, either an animal transport cart or a disposable cart cover (at the exits of the facilities) OR a disposable animal transport bucket or “takeout box” (at the exits of the facilities) must be utilized. Federal regulations forbid animals from being housed in laboratories for more than 12 hours without approval from the IACUC.

Upon returning to the animal facility, the cart cover should be discarded, and one of the following options should be performed:

- **Natural Science Center (NSC):** Empty cages should be placed on the provided cart in the foyer of the animal facility.
- **Petit Science Center (PSC):** Empty cages should be sprayed down at the animal facility entrance with appropriate disinfectant and placed on the receiving table immediately inside the dirty cage wash facility.
- **NSC and PSC:** Exteriors of occupied cages should be sprayed down with the appropriate disinfectant at the facility entrance (unless DAR management has
approved the cage exteriors do not need to be sprayed down as is the case with some Syrian hamster cages) and returned to the appropriate housing room and then labeled with a “Cage Returned from Lab” card (these labels are located at the facility entrance by the lab coats). These cages will then be changed into new cages by DAR Staff. In cases of disposable transport buckets and “takeout boxes”, the exteriors should be sprayed down with the appropriate disinfectant at the facility entrance and returned to the appropriate housing room. The animal should then be transferred to its home cage. The disposable bucket or box should be thrown away in the housing room.

- In all cases, the used transport cart must be sprayed down with appropriate disinfectant (including wheels and shelves) and placed immediately inside the dirty cage wash facility

**Animal Transport off the GSU Campus:**
The DAR maintains a climate-controlled van that must be utilized to transport animals to and from the GSU campus. It is necessary to take GSU driver certification training in order to drive the van. Please contact the DAR Office at 404-413-3560 for assistance in scheduling attendance at a driver certification course and to schedule use of the van and the associated procedures to be followed.

4. **Food or Water Restriction**
Ethical principles and federal regulations dictate that animals be fed a wholesome and nutritionally adequate diet taking into consideration the species being used and individual animal characteristics (e.g. age, strain, housing type, etc.). However, it is recognized that the restriction of food or water intake may be required for the conduct of some experimental studies. The restriction process may entail scheduled access to food or water sources, so an animal consumes as much as desired at regular intervals, or a plan in which the total volume of food or water that is consumed is strictly monitored and controlled.

Restriction of food and/or water must be scientifically justified in the IACUC protocol and approved by the IACUC prior to initiation of the research. Because food and/or water restriction may be conducted for a variety of reasons, the IACUC will consider the maximum period and severity of restriction on a case-by-case basis with reference to the welfare of the animals (including consideration of the age, species and natural biology of the animal) as well as the goals of the particular study. The minimum period and severity of restriction needed to achieve the desired objective of the restriction should be elucidated. A monitoring plan to assess potential adverse consequences of the restriction as well as specific criteria for intervention and removal of an animal from a study must be clearly described in the IACUC protocol. Any animal under food and/or water must be observed as frequently as necessary, but at a minimum of daily, by personnel trained to evaluate the animal’s health and wellbeing. The weights of most animals approved for food restriction must be measured and recorded at no less than weekly intervals, and smaller animals such as mice, or young animals that are growing may require more frequent weighing. The weights of most animals approved for water restriction must be measured and recorded at no less than 24-hour intervals, or a record of daily water intake must be maintained to ensure adequate hydration. Written records should be maintained for each animal undergoing food and/or water restriction to
document any monitoring parameters as elucidated in the IACUC protocol such as body weight, fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal from the study. The records must be available for inspection by the IACUC, DAR veterinarians, and any regulatory or accrediting agencies.

If an animal under food and/or fluid restriction loses more than 15% of its body weight (compared to its pre-restriction weight), its food and/or fluid intake should be increased immediately as appropriate until the animal regains its normal weight (+/- 15%). The weight of any animal that has lost 15% of its body weight should be measured daily to ensure that further body weight is not lost. An exception to the 15% weight loss policy may be allowed if a DAR veterinarian determines that this weight loss does not endanger the health of the animal (for example, if the animal is adequately hydrated and was initially overweight). Additionally, greater than 15% weight loss can be allowed if the IACUC has given prior approval for greater weight loss in a particular animal study. Food and/or fluid restriction in young animals that are still growing will be considered on a case-by-case basis, as weight loss is not appropriate for animals that should be growing.

In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended. Food and water consumption are interdependent, but species differ in their circadian or other patterns of drinking and their response to food restriction. Unless specific protocols require exemption, allowing most laboratory animal species to feed at least once per day is consistent with standards of humane care and is required for species covered by USDA regulations. Constant access to water typically is provided under food control regimens, but requirements of the species and the scientific protocols may require different patterns of access. Conversely, water-deprived animals often have non-restricted access to food, but investigators should be aware that most food consumption occurs only when water is available. Water should be available long enough to maintain sufficient food intake. Animals tolerate food restriction physiologically better than water restriction, so food restriction should be used if possible. Fluid reinforcers often have advantages, however, such as in procedures that must control the position of the subject’s head or limit jaw movements. When water, sweet drinks, or fruit-flavored drinks are used as a reinforcer, access to water outside the experimental session needs to be controlled. Determining parameters of water restriction, including especially the period(s) of access during the day that do not produce dehydration or excessive weight loss requires careful consideration and sensitivity to the species. When this is done, animals need not be at risk. Careful observation of behavior, regular clinical monitoring of the animal’s health and records of measures taken are critical for ensuring successful application of fluid control procedures.

If food and/or water is being withheld, please the DAR management staff to obtain the appropriate cage label which will allow the cage to be conspicuously labeled as under food and or water restriction.

5. Housing Rodents on Wire Floors

When given the choice, rodents prefer solid floors (with bedding) to grid or wire-mesh flooring. The Guide recommends that animals should have adequate bedding substrate and/or structures for resting and sleeping. For many animals (e.g., rodents) contact
bedding expands the opportunities for species-typical behavior such as foraging, digging, burrowing, and nest building (Armstrong et al. 1998; Ivy et al. 2008). Moreover, it absorbs urine and feces to facilitate cleaning and sanitation. If provided in sufficient quantity to allow nest building or burrowing, bedding also facilitates thermoregulation (Gordon 2004). Breeding animals should have adequate nesting materials and/or substitute structures based on species-specific requirements solid bottom caging with bedding be used preferentially for rodents. There is some evidence that limb pathology has been associated with prolonged housing of rodents on wire mesh floors. The IACUC is expected to address this issue during protocol review, and if you want to house animals on wire mesh flooring, you will be asked to provide a scientific justification on the protocol form.

Some toxicology projects in rodents are performed on wire mesh floors so that animals do not remain in contact with metabolites in urine and feces. Rodents in metabolism cages must usually be on wire mesh floors so that urine and feces can be collected under the cage.

6. Prolonged Physical Restraint
The Guide also has special language addressing prolonged restraint of animals while they are conscious. In general, restraint for all animals should be the least restrictive and for the shortest time necessary to complete research objectives.

Prolonged restraint, including chairing of nonhuman primates, should be avoided unless it is essential for achieving research objectives and is specifically approved by the IACUC. Systems that do not limit an animal’s ability to make normal postural adjustments (e.g., subcutaneous implantation of osmotic mini-pumps in rodents, backpack-fitted infusion pumps in dogs and nonhuman primates, and free-stall housing for farm animals) should be used when compatible with protocol objectives. Animals that do not adapt to necessary restraint systems should be removed from the study. When restraint devices are used, they should be specifically designed to accomplish research goals that are impossible or impractical to accomplish by other means or to prevent injury to animals or personnel.

The following are important guidelines for restraint:
- Restraint devices should not be considered a normal method of housing, and must be justified in the animal use protocol.
- Restraint devices should not be used simply as a convenience in handling or managing animals.
- Alternatives to physical restraint should be considered.
- The period of restraint should be the minimum required to accomplish the research objectives.
- Animals to be placed in restraint devices should be given training (with positive reinforcement) to adapt to the equipment and personnel.
- Animals that fail to adapt should be removed from the study.
- Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.
- Veterinary care must be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates the temporary or permanent removal of the animal from restraint.
The purpose of the restraint and its duration should be clearly explained to personnel involved with the study.

7. Mouse Breeding Trios
Georgia State University IACUC recognizes and supports that this is an established and appropriate method of breeding mice. In keeping with the housing space provisions as indicated in the 8th Edition of the Guide for the Care and Use of Laboratory Animals, the GSU IACUC has adopted the following policy with respect to mouse breeding trios: The investigator will assure that, if three adult mice are in the cage, no more than 12 pups total will also be in the cage at any given time. Should the number of pups in the cage exceed this amount then 1 female and her litter will be moved to a separate cage.

8. Social Housing of Social Species
Social housing of social species will be considered by the Georgia State University as the default method of housing unless otherwise justified based on social incompatibility, veterinary concerns regarding animal well-being, or scientific necessity approved by the IACUC. When necessary, single housing of social animals should be limited to the minimum period necessary and, where possible, visual, auditory, olfactory and, depending on the species, protected tactile contact with compatible conspecifics should be provided. In the absence of other animals, additional enrichment should be offered, such as safe and positive interaction with the animal care staff, as appropriate to the species of concern; periodic release into larger enclosures; supplemental enrichment items; and/or the addition of a companion animal in the room or housing area. This policy and exceptions for single housing will be reviewed on a regular basis and approved by the IACUC and/or the veterinarian.

a. Social Housing of Laboratory Animals
Definitions
A. A social species is defined as any species known to naturally live and interact with conspecifics (animals of the same species). The majority of laboratory animals housed at the GSU are considered social species, including but not limited to the following: most rodents (mice, rats, guinea pigs), rabbits, ferrets, nonhuman primates, and aquatics (frogs and fish).
B. Social housing is defined as housing social species in compatible pairs or groups with additional visual, auditory, olfactory, and/or tactile contact of conspecifics housed within the same room.
C. Single housing is defined as housing an animal in a primary enclosure by itself with additional visual, auditory, olfactory, and/or tactile contact of conspecifics housed within the same room.
D. Solitary housing is defined as housing an animal in a primary enclosure by itself in the absence of any other animals in the same room.
E. Social experience consists of a broad spectrum of possible social situations and interactions that may vary based on the species, health status, caging or housing systems available, or experimental use of the animal. These experiences can include:
   1. Full time social housing characterized by unrestricted contact with conspecifics in the same primary enclosure.
2. Part time social housing characterized by unrestricted contact with conspecifics for a defined time period and/or defined frequency. Examples include overnight social housing, intermittent social housing permitted between animal studies, and intermittent direct contact with conspecifics in a group setting for a defined time period.

F. Single housing based on social incompatibility, veterinary concerns, or scientific necessity but supplemented by limited or protected social contact through a mesh panel, grooming bars or other type of perforated barrier on either a part or full time basis.

Full time social housing is the preferred and expected method for housing social animals unless otherwise justified based on scientific rationale outlined in the IACUC approved animal care and use protocol, social incompatibility, or veterinary medical or animal welfare concerns.

Single housing of social animals, when necessary, should be limited to the minimum period required and provide a combination of visual, auditory, olfactory, and tactile contact of conspecifics when possible. Single housing in the absence of other animals (solitary housing) requires the provision of additional enrichment to provide for a social experience, which may include but is not limited to positive interaction with animal care personnel, periodic supervised access to larger enclosures, and supplemental enrichment items approved by DAR management.

Exemptions from social housing require scientific justification outlined in the animal care and use protocol approved by the IACUC. Protocol related exemptions must be reviewed and approved by the IACUC on an annual basis. Protocol related exemptions must also be identified on the semi-annual report submitted to the Institutional Official.

The IACUC may also grant program wide social housing exemptions. The following are IACUC approved program wide social housing exemptions:

1. Single housing intact male rabbits due to aggressive behaviors commonly observed when pair or group housed.

2. Standard practices in breeding colony management that result in the need to periodically single house animals, including:
   a. Single housing breeder males between mating with females,
   b. Single housing pre-parturient females,
   c. Single housing animals of either sex at weaning when the litter makeup contains a single male and/or a single female at the time of weaning.

3. Standard practices in managing surgery or other technical procedures including:
   a. Single housing animals for fasting prior to surgery or other procedures that require general anesthesia,
   b. Single housing animals for up to 14 days for post-operative recovery and observation. The need to single house animals for greater than 14 days post-operatively must be outlined in the IACUC approved protocol.
4. The unavailability of another socially compatible animal due to:
   a. Aggression or incompatibility
   b. Research attrition

A DAR Veterinarian may exempt animals from social housing on an individual basis due to incompatibility or for veterinary medical and/or animal welfare concerns. Veterinarian exemptions must be documented in the individual animal's clinical record every 30 days unless the exemption is permanent.

b. Social Housing of Non-Human Primates
Non-human primates at the LRC are housed as “full time social housing” or “part time social housing”.

- Full time social housing characterized by unrestricted contact with conspecifics in the same primary enclosure.
- Part time social housing characterized by unrestricted contact with conspecifics for a defined time period and/or defined frequency. Examples include overnight social housing, intermittent social housing permitted between animal studies, and intermittent direct contact with conspecifics in a group setting for a defined time period.

Capuchins
All capuchins have full time social housing with socially compatible conspecifics. They have access to both outdoor and indoor primary enclosures at all times except during research testing, inclement weather, cleaning or cage repair and modification.

Chimpanzees
All chimpanzees have full time social housing with socially compatible conspecifics. They have access to both outdoor and indoor primary enclosures at all times except during research testing, inclement weather, cleaning or cage repair and modification.

Rhesus Macaques
The rhesus macaques have part-time social housing. Specifically, each rhesus macaque’s home cage (a Britz “play cage”) is connected to a “tunnel system.” At any time, a macaque can leave its home cage and enter a section of the tunnel system as an expansion of its cage. Each tunnel system is connected to an outdoor turnout. The tunnels have doorways which allow control of physical contact between compatible pairs. The tunnel doorways are open once a day for a couple of hours to allow compatible pairs to have access to one another in outdoor turnouts. All rhesus macaques have continuous visual and vocal access to other rhesus macaques.

c. Exemptions and Exceptions
Single housing of social species should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being (Guide, p. 64). Exemptions to this policy may be evaluated based on the experimental and/or clinical requirements of the particular animal.
• Scientific-related social housing exemptions must be outlined in the animal care and use protocol approved by the IACUC. Protocol related exemptions must be reviewed and approved on an annual basis by the IACUC, consistent with the annual continuing review of the protocol (AWR 3.81 (e)(2)).

• Veterinary-related concerns will be initiated by the Attending Veterinarian (AV) and focus on temporary single housing due to the NHP's health or condition, or in consideration of its well-being. Unless the basis for the exemption is a permanent condition, the exemption must be re-evaluated and documented by the AV at least every 30 days (AWR 3.81 (e)(1)). Examples of exemptions for veterinary reasons are found below:

• If a nonhuman primate exhibits vicious or overly aggressive behavior (AWR 3.81 (a)(1)) it will be singly-housed for the safety of personnel and other NHPs. Future grouping attempts with new partners may be made with aggressive animals, at the discretion of the AV.

• If a nonhuman primate is debilitated as a result of age or other conditions (AWR 3.81 (a)(1)), then it may be singly-housed to preserve the welfare of that animal. "Debilitated" may include chronic health issues, excessive stress or distress, or other conditions as determined by the AV.

• Nonhuman primates that have or are suspected of having a contagious disease must be isolated from healthy animals in the colony as directed by the AV (AWR 3.81 (a)(2)). When/if the concern for the contagion has passed, the AV will reconsider group-housing.

If individual animals cannot be co-housed successfully, singly-housed animals will have visual, auditory and olfactory contact with at least one social partner, unless exempt for research purposes (IACUC) or clinical care (AV). The Language Research Center environment also affords considerable positive human interaction provided by laboratory and animal care staff as part of the psychological and environmental enrichment. In addition, the research programs at the Language Research Center involve a myriad of non-invasive cognitive tasks in which a given animal can choose whether or not to participate; the tasks themselves are a robust form of environmental enrichment.

9. **Mouse Tumor Burden**

These guidelines allow GSU researchers, veterinary staff, and animal care staff to objectively evaluate the health and welfare of mice used with experimentally-induced solid tumors and determines when euthanasia might be warranted to alleviate pain and distress associated with these solid tumors. In general, solid tumors are induced by the administration of chemical carcinogens or viruses, inoculation with tumor cell lines, transplant of tumor fragments, or genetic manipulations.

With respect to mouse studies involving experimentally-induced solid tumors, it is typically necessary that the mice utilized experience tumor growth and possibly metastatic disease. These processes can ultimately be very debilitating for the mouse. In order to maximize study data acquisition and minimize animal pain and distress, the
general health and welfare of test subjects need to be continually and comprehensively assessed and documented as the tumors and associated disease progress.

Euthanasia may be warranted in tumor-bearing mice when such mice incur one or more health issues (e.g. from the list below or otherwise). In particular, the research staff, in conjunction with the veterinary staff, can work together to determine when an animal should be euthanized vs. rendered supportive therapy and/or monitored closely. The GSU IACUC has adopted the following criteria as objective assessments necessitating empirical euthanasia of the tumor-bearing mouse.

1. Animals with tumors will be weighed twice a week, with at least two days, but no more than four days, between each weighing.

2. Body Condition (See Appendix 1 for Guidance): A body condition score of 1 requires euthanasia.

3. Weight loss of >20% (or a deviation from an age matched control or a standard growth curve by more than 20% as it pertains to animals beginning the study as neonates or juveniles) requires euthanasia. Note: all animals should have bodyweights recorded at the initiation of the tumor study and these weights recorded in the Laboratory Animal Care Record located in the animal housing room.

4. Tumor Size: If, in a mouse, a single tumor is larger than a dime (spherical), or the aggregate of multiple coalescing tumors is larger than a dime then euthanasia is required.

5. Other clinical presentations, such as those delineated below, warrant special attention as they indicate a diminished health status that may result from an increasing tumor burden and metastasis. When such are seen they should be noted in the Laboratory Animal Care Record and brought to the attention of the veterinary staff. Determinations regarding euthanasia as it relates to the clinical presentations below will be made by the veterinarian on a case-by-case basis.
   - Weight loss (or a failure to grow, if young mice) and decreasing body condition
   - Severe diarrhea
   - Progressive dermatitis
   - Rough hair coat, hunched posture, lethargy, and recumbency
   - Respiratory-associated symptoms such as labored breathing, coughing, and nasal discharge
   - Icterus/Jaundice
   - Hemorrhage from any orifice
   - Neurological signs such as circling or ataxia
   - Self-trauma
   - Tumor interference with activities such as ambulation and/or food and water consumption
   - Ulceration and necrosis of visible tumors

10. **Approved Housing Areas**
The following locations at Georgia State University have been approved by the IACUC for housing animals longer than 12 hours:
11. Capture of Escaped Rodents

Precautions should be taken to prevent/minimize the occurrence of an escaped rodent. When opening a rodent cage, always be sure to replace the cage top if more than one rodent is in the cage so as to prevent the opportunity for the remaining rodent to escape. Always be aware of the number of rodents in the cage prior to the cage being opened and verify this number when one is finished with the cage. This policy will be posted on the inside of each animal room containing rodents and training on this policy will occur during the conduct of the animal facility orientation process.

Procedure to Capture an Escaped Rodent

a. Any rodent that escapes from its cage or work area and is found running loose within a room/hallway is considered an escaped rodent.

b. During initial escape, try to capture and isolate the rodent by the following methods:
   
   i. Catch the rodent by hand: *Personnel working in ABSL-2 or ABSL-3 Biocontainment areas must wear Kevlar gloves in addition to the normally prescribed PPE in these areas when hand catching rodents to minimize the risk of personnel exposure via a bite or scratch. The DAR will provide Kevlar gloves in the ABSL2 and ABSL3 suites for this purpose.
   
   ii. Cup cover method: Use a cup or a similar object to cover and isolate the rodent. One can then slide a paper or similar object under the cup to form a “lid” and thus contain the rodent for placement into a rodent cage.
   
   iii. Traps: If unsuccessful with hand capture or the cup method, humane traps or snap traps can be placed by DAR management to capture the escaped rodent.

c. If capture is successful, place the animal in a clean/lidded rodent cage by itself. Place a cage card on the cage and label the card as “Animal Escaped” and date of the escape. If known label the cage with the PI and protocol number.

d. Always notify DAR management of the escaped rodent even if the rodent is captured.

e. If the rodent is found without prior report of an escape, the DAR management team will communicate with the appropriate Principal Investigators (PIs) and research staff to try to identify the rodent through the use of ear markings, coat color, location in which the rodent was found/seen, and/or the absence of a rodent from a given animal cage.

f. Upon being notified of an escaped rodent or otherwise finding an escaped rodent, a member of the DAR management team will notify the Biological Safety Officer and the IACUC office of the escaped rodent.
g. If the animal is able to be associated with a particular Principal Investigator (PI), the veterinarian will discuss the final disposition of the captured rodent with the PI or associated research staff.

h. If the animal is unable to be associated with a particular PI, the veterinarian will determine the final disposition of the captured rodent. Particularly if it is not possible to associate the escaped rodent with a particular PI, the final disposition is typically euthanasia of the rodent at which time tissues may be collected (serum and formalin-fixed tissues). These samples will be held until a decision is made by the veterinarian, the Biological Safety Officer, and the IACUC Chair that it is either ok to discard these tissues or that the tissues are to be submitted for testing.

For animals housed in the ABSL-3 Biocontainment area, the DAR staff conducts a “head count” of the rodents contained in this area on a daily basis. Further, the researchers working in this area are required to document all subtractions (via a spontaneous death or euthanasia). Accordingly, the DAR management team is able to readily ascertain if an animal is missing from this area.

12. Overcrowded Cages

Cages are considered overcrowded either because litters need to be weaned or there are too many post-weanling and/or adults in one cage. Please note the maximum numbers of mice housed per cage are as follows:

- 5 adult mice
- Pair housed mice (1 female, 1 male): unrestricted number of pups
- Breeding trios (2 females, 1 male): maximum of 12 pups per cage

If a cage is overcrowded, it will be flagged with a red “Overcrowded Cage” card and an email notification will be sent to the animal user. The animal user will then have 48 hours to resolve the overcrowded cage. If the overcrowded cage is not corrected within 48 hours, the DAR animal care technician will separate animals into appropriate cages (a technician fee will apply).

Please note that while it is the Division of Animal Resources (DAR) responsibility to correct overcrowding, it is advised not to rely upon DAR as animal users have the best knowledge of genotyping and pedigree information which is vital.

These maximum numbers of mice per cage are based on recommendations from the “Guide for the Care and Use of Laboratory Animals” (The Guide). Exemptions to this policy are permitted only upon specific IACUC review and approval. If exemptions apply, please note it is the responsibility of the animal user to detail the exemption in the “Special Housing or Husbandry Requirements” section of the “Animal Requisition” form.

13. Quarantine

a. Animals arriving from non-commercial vendors will be quarantined.

b. Animals under quarantine must remain in the animal housing room and associated ante-room until the time that they are released from quarantine.

c. Animals under quarantine will be housed in either ventilated cages or static micro-isolator cages.
d. Upon arrival quarantined mice will receive 3 microliters of Moxidectin per mouse to empirically treat for fur mites. At the discretion of the veterinarian, a second dose may be given 10 days after the first.

e. Quarantined animals will undergo a cage change on day 6 after arrival.

f. On day 7 after arrival, the samples will be obtained for quarantine testing (see veterinarian for specific samples to collect and testing profiles to run as this will vary on a case by case basis). Also at this time the animals will receive fenbendazole-impregnated rodent diet or fenbendazole-impregnated Napa nectar. The fenbendazole-impregnated diet or fenbendazole-impregnated Napa nectar will be administered for a period of six weeks to empirically treat for pinworms. The six week treatment regimen may be discontinued at the discretion of a DAR veterinarian commensurate with pinworm testing results.

g. Quarantine-housing rooms must be entered last of all facility rooms on campus, unless door signage directs otherwise.

h. Follow signage on the door as to what personal protective equipment (PPE) is necessary.
   i. PPE includes: shoe covers, hair bonnet, gloves, disposable gown, and face mask.

i. All PPE must be disposed of in a biohazard container in the anteroom. Disposable gowns may be reused and hung on a hook inside the quarantine housing room.

j. Cages under quarantine must be opened and changed consistent with DAR SOP #44 entitled "Change outs for Rodents Using the Biological Safety Cabinets- All BSL2, BSL2+, and Quarantine Cages."

k. Multiple orders of quarantine animals may be housed in one quarantine room at the same time. A DAR veterinarian will designate the cage change order.

l. Animals in quarantine will be tested under the following paradigm (which may be modified by a DAR veterinarian) with samples collected seven days after arrival:
   i. Pinworm PCR: Submit 1 fresh fecal pellet from each cage or animal. Up to 10 fecal pellets can be pooled to reduce costs. Fecal pellets for PCR evaluation should be collected with clean gloves or sterile forceps and placed in individually labeled sterile containers. The veterinarian will determine the number of samples to collect. Follow the fecal pellet collection method specified below.

   ii. Fur mite PCR: Two options are available for testing mice or rats for fur mites: Pelt swabs or cage swabs. The veterinarian will determine the number of samples to collect. Follow the pelt swab collection method or the cage swab collection method below.

   iii. Helicobacter and Viral PCR: The veterinarian will decide the assay to run after reviewing the incoming animal health report from the shipping institution. Follow the fecal pellet collection method specified below.

   iv. Opti-Spot Viral and Bacterial Serology: The veterinarian will determine the number of samples to collect. Follow the Opti-Spot blood collection method specified below.
v. Additional testing may be determined by the veterinarian (e.g. Pasteurella pneumotropica oral culture swab, gross pathology, etc.).

1. **Fecal pellet collection method:**
Fecal pellets for PCR evaluation should be collected with clean gloves or sterile forceps and placed in individually labeled sterile containers. If testing of individual mice is required, submit fecal pellets from each mouse. Two to three pellets per animal are adequate. NOTE: fresh fecal pellets either obtained directly from mice or from soiled cages within 24 hours of the most recent cage change are recommended for testing. If multiple animals of the same microbiologic status are to be evaluated, up to 10 fecal pellets can be pooled and tested as one sample. If fecal pellets from multiple animals are to be collected, gloves should be changed or forceps should be replaced between animals to prevent cross contamination. Alternatively, forceps can be wiped clean and immersed in dilute bleach (10%) solution for 10 minutes prior to reuse. Fecal pellets for identification of Helicobacters and MNV should be shipped by an overnight carrier, but do not need to be chilled or frozen. Fecal pellets for identification of other viruses should be shipped to IDEXX by an overnight courier at room temperature or with cold pack(s).

2. **Pelt swab collection method:**
Using a sterile, dry flocked swab, thoroughly swab against the direction of hair growth of the head, rump and inguinal area of one or more mice or rats in the cage. Up to 10 animals can be swabbed using one swab, or use a new swab for each animal combining up to 10 swab tips per sample. Insert the swab halfway into a sterile microcentrifuge tube, close the microcentrifuge tube lid against the swab shaft and pull down on the swab shaft to break the shaft. The tip end will fall into the tube and the tube can be capped. Plastic shaft swabs are preferred over wooden shaft swabs as they break cleanly. Swabs can be shipped by overnight courier at room temperature or on ice packs.

3. **Cage swab method:**
Using a sterile, dry flocked swab, swab the inside perimeter of empty soiled mouse or rat cages. ANIMALS SHOULD HAVE OCCUPIED THE CAGES FOR AT LEAST 4 DAYS PRIOR TO USING THIS METHOD. Swab the inside perimeter of the cage at the level of the bedding. Up to 10 cages can be swabbed using the same swab. Insert the swab halfway into a sterile microcentrifuge tube, close the microcentrifuge tube lid against the swab shaft and pull down on the swab shaft to break the shaft. The tip end will fall into the tube and the tube can be capped. Plastic shaft swabs are preferred over wooden shaft swabs as they break cleanly. Swabs can be shipped by overnight courier at room temperature or on ice packs.

4. **Opti-Spot blood collection method:**
Opti-spot serology samples will be collected via venipuncture. The whole blood sample will be placed on an IDEXX strip/card, there should be
enough blood to saturate the diameter of the circle on the strip. The blood should be allowed to dry for at least an hour. The upper tab over the dried spot is folded and the card/strip is placed in a waterproof plastic bag with the silica gel desiccant pack. The sample is shipped at room temperature or with cold pack(s).

m. Animals in quarantine may not be bred unless special approval is granted by the veterinarian.

n. Researchers do not have access to quarantine unless special approval is granted by the veterinarian. If approval is granted the researcher would be escorted by DAR.

14. **Mouse Rectal Prolapse**

Rectal prolapse is the protrusion of the rectum from the area just below the tail. Mice are particularly susceptible to rectal prolapse because they have a very short rectum where the descending colon enveloped in serosa extends almost to the anus. Therefore, a rectal prolapse in mice can occur simply because of straining during bowel movement or during the process of giving birth.

A high incidence of rectal prolapse in a colony of immune deficient mice is often linked with *Helicobacter* species infections. Other strains of bacteria are also implicated but are found less commonly. Diarrhea, intestinal mass/tumor, or proliferative/inflammatory typhlitis and/or colitis could also increase the incidence of rectal prolapse in a colony. Some genetically engineered mice with immune system alteration (e.g., IL-2 and IL-10 deficient, TCR α, β, γ positive, MHC-II and Ga-i2 deficient) have rectal prolapse with colitis in the absence of (diagnosed) helicobacteriosis.

There is no specific treatment for rectal prolapse in mice. On initial exam the DAR veterinary staff will apply petroleum jelly or hemorrhoid cream to the area. This will not treat the problem in any way. Rather it is simply an attempt to soothe the lesion until the mouse is used or euthanized. Euthanasia within 24 hours of notification is required for animals with rectal prolapse that meet one or more of the following criteria:

- Prolapsed tissue protruding ~4 mm or more
  - Depression
  - Ruffled fur
  - Necrotic prolapsed tissue
  - Hunched posture
  - Weight loss of greater than 20%

If euthanasia is not indicated, petroleum jelly or hemorrhoid cream must be applied to the rectal prolapse daily as the rectal tissue can become dry and necrotic. Please note there will be a technician fee for daily application of petroleum jelly or hemorrhoid cream. In addition, the animal’s body condition and weight must be assessed at least weekly and the animal euthanized if/when its condition deteriorates.

Rectal prolapse is easily detected when mice are picked up by the tail. However rectal prolapse and uterine prolapse are not always easy to distinguish from each other. Check
closely to determine through which opening the organ is protruding. If the prolapse is uterine, the general recommendation is euthanasia as the prognosis is poor.

15. Rodent Genotyping and Identification

Rodent genotyping is commonly performed using tail snipping or ear punch techniques, and less frequently using toe-clipping. Tail tipping involves cutting the tip of the tail of small rodents for the purpose of obtaining a tissue sample for genetic analysis. Ear punch tissue sampling is a less invasive technique. Further, genotyping via the use of a 2mm ear punch sample appears to yield results comparable to that produced via genotyping via tail snipping. In addition, the ear punch method can also be used for animal identification. Toe-clipping involves cutting the distal half of a digit to obtain tissue sampling for genotyping and/or for identification; it is generally considered to be the most invasive procedure of the 3 forms of genotyping, and therefore it is less frequently used. Accordingly, the use of the ear punch method is encouraged.

Procedural Descriptions:

Tail Snip:

Pain perception of tail clamping in rats does not start to develop until 12 to 14 days of age1, so performing tail biopsy earlier in rodents may cause less pain. When performed properly in adult mice it causes only minimal or transient pain and distress, and induces no more “physiological impact” (change in heart rate, body temperature, or activity level) than restraint of the animal for the procedure.

Guidelines for Tail Snip

For mice and rats 12 days of age and younger: Tail biopsies should preferably be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, bleeding is minimal, and anesthesia is not required.

For mice and rats 13-21 days of age: Based on the physiological impact and rodent pain ontogeny studies, investigators are required to utilize an analgesic regimen such as immersion of the tail in ice cold ethanol for 10 seconds, topical application of ethyl chloride spray, by injection of an analgesic (e.g. carprofen, buprenorphine, etc.) at least 20 minutes prior to the conduct of the procedure, etc.

For mice and rats greater than 21 days of age: The use of an analgesic or general anesthetic is required prior to collection of tissue.

For mice and rats greater than 35 days of age: The use of a general anesthetic is required. To conduct the procedure, manually restrain the mouse or rat between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc.). With sterile scalpel, razor blade, or scissors cleanly excise the distal 2mm (maximum 5 mm) of the tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should be enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. If small amounts of DNA are required, investigators should consider taking only 2 mm of tail. If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also disinfect the work surface on which the tail is placed between animals.
The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. If needed, apply digital pressure, silver nitrate, or some other means of hemostasis. Each animal will typically only be genotyped once. However, if a second tail snip is needed (e.g. due to a technical error with the PCR, etc.) then only one additional tail snip is permissible and the total amount of tail removed will not exceed 5 mm (first and second snip combined).

**Ear punch:**
- Ear punches can be performed on any age of mouse beginning at about two weeks of age.
- Manually restrain the mouse.
- A 2 mm ear punch device is used to carefully punch the ear and allow for the removal of a sufficient sample of ear tissue for genotyping.
- The ear punch device will be sanitized (wipe with 70% alcohol) between mice.
- Bleeding from the site of the ear punch is uncommon. If bleeding is seen, hemostasis will be ensured by applying gentle pressure to the collection site for 1 minute. Blood cauterization (e.g. via application of a silver nitrate stick) may be used as indicated.

**Toe-clipping:**
- Toe clipping of neonatal mice provides permanent identification and genotyping material. The 8th edition of The Guide for the Care and use of Laboratory Animals states the use of toe-clipping as an identification method, should only be used when no other individual identification method is feasible. The Guide also states that “It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age.”
- Toe-clipping technique can be applied to animals up to 7 days of age without anesthesia, and should not be used on animals after 7 days of age.
- Toe-clipping must be scientifically justified and used as both an identification method and for genotyping material in order to obtain IACUC approval.
- The distal half of each toe is transected with a sterile scalpel blade or surgical scissors.
- A maximum of 2 digits per paw are permitted to be removed.
- Scissors or scalpel blade will be sanitized (wipe with 70% alcohol) between mice.
- Upon completion, hemostasis will be ensured by applying gentle pressure to the collection site for 1 minute. Blood cauterization (e.g. via application of a silver nitrate stick) may be used as indicated.

**Rodent Identification**
Rodent identification can be accomplished via several techniques including ear punching, ear tagging, ear snipping, tattooing, micro-chipping, permanent marker, or toe-clipping. All listed forms of identification are acceptable.

**Procedural Descriptions:**
Ear punching:
• See above for description.

Ear tagging:
• Ear tagging can be performed on rodents weaning age or older animals.
• Disinfect (with 70% alcohol) or sterilize the metal ear tag.
• Manually restrain the rodent.
• An ear tag device is used to apply the ear tag to the pinna.
• Seldom are there complications with tagging such as feet getting caught in the ear tag, or the development of severe irritation around the ear tag site.

Ear Snipping:
• This practice of tissue collection requires the removal of a 2-3mm wedge of tissue from the ear pinna with sharp scissors.
• Ear snips should be taken from animals between the ages of 8 and 12 days of age because there is a lack of nervous system development, bleeding is minimal, and anesthesia is not required.
• After day 12, local anesthesia is required because of the developed vascular and nervous system.

Tattooing:
• Manually restrain the rodent.
• A permanent mark is made on the tail, toes, ears, or possibly foot pads by using a needle or appropriate micro-tattooing device.

Micro-chipping:
• Ear tagging can be performed on rodents weaning age or older animals.
• Manually restrain the rodent.
• Inject a small microchip transponder subcutaneously between the scapulae of the rodent.
• The microchip is detected by use of a reader.

Permanent marker:
• Permanent marker technique is used for temporary identification.
• Manually restrain the animal.
• Apply the marker to the appropriate location.
• This method is non-invasive.

Toe-clipping:
• See above for description.

16. Weaning Mice
This is not mandatory but recommended for all researchers who maintain breeding colonies of genetically engineered mice (GEM) at Georgia State University. The purpose is to minimize adverse events associated with housing newly weaned GEMs. Some strains of GEMs have increased complications (i.e. illness, mortality) during weaning, as some strains are more “fragile” than other strains. GEMs with weaning complications will be referred to as GEMWWC (genetically engineered mice with weaning complications).
The GEMWWC strains will be identified by DAR and these strains will be required to adhere to the mouse weaning policy. DAR will notify respective labs, if they have any GEMWWC strains.

a. **Training Requirements**

Hands-on training is required for personnel who breed and wean strains of GEMWWC. Training will be provided through the Division of Animal Resources (DAR).

b. **Delineation of Responsibilities**

It is expected that both laboratory and DAR staff will provide appropriate care to all research animals on an ongoing basis. However, in situations where laboratory staff are breeding and weaning strains of GEMWWC, the laboratory is responsible for correctly setting up weanlings in new cages. Once these animals are weaned, primary responsibility for their daily care shifts to the DAR animal care staff. However, as is the case in general, laboratory personnel are responsible for reporting to DAR any animal care issues that they observe as they are conducting other procedures with the research animals.

c. **Weanling Cage Set Up**

- **Cages**
  - To prevent overcrowding, mouse litters are to be weaned between 19 and 25 days postpartum. Exceptions are allowed, as indicated under the Special Circumstances section below. Regardless, it is the responsibility of the investigator to adhere to the maximum number of mice housed per cage policies:
    - 5 adult mice
    - Pair housed mice (1 female, 1 male): unrestricted number of pups
    - Breeding trios (2 females, 1 male): maximum of 12 pups per cage
  - All weanling animals must be placed in a clean, standard rodent cage with food and water.

- **Water**
  - A fresh water bottle must be provided.

- **Food**
  - The food hoppers must be filled with chow appropriate for the particular strain.
  - If breeding and weaning strains of GEMWWC, a portion cup must be placed in the bottom of the cage containing moistened rodent chow. The cup should be prepared by adding an excess of water to a few food pellets so that they become softened for easy consumption.

d. **Verification by laboratory and DAR animal care personnel**

- Once the weanling cage is set up, the laboratory member must initial and date a “Newly Weaned” card and insert it with the standard cage card
such that it is visible to DAR animal care staff. These cards are provided by DAR (see Appendix A).

- By 5:00 pm of the day following initial set-up, DAR animal care staff must independently verify that the weaning cage is set up correctly and that the animals appear healthy. If this is the case, then the DAR animal care staff initial and date the weaning card.
- If the DAR animal care staff find that the cage has not been set up properly, they will correct the deficiencies upon discovery and initial and date the weaning card. They will then contact the lab and document the corrective care. A technician fee will apply for this additional care.

e. **Subsequent Care**

- Post-weaning, DAR animal care staff will monitor the animals on a daily basis.
- “Newly Weaned” cards will be removed at the discretion of DAR animal care staff (typically at the time of cage change).

f. **Special Circumstances**

The procedures indicated above are recommended for all genetically engineered mice (GEM) but required for genetically engineered mice with weaning complications (GEMWWC). It is understood that some strains of genetically engineered mice might require additional care, in those circumstances it is considered best practice for the laboratory staff to work with the veterinarian and DAR animal care personnel to develop specific programs of care for these strains. In these instances, the standard of care must be detailed in the IACUC protocol and the animal user should detail any special circumstances in the “Special Housing or Husbandry Requirements” section of the “Animal Requisition” form. Charges may apply if additional care is required. Issues to consider when developing a standard of care for a fragile strain/line include but are not limited to the following:

- Age of weaning past 25 days of age
- Addition of supplemental food/water sources such as hydration or nutrient gels

J. **Additional Considerations Pertaining to Protocols**

1. **Antibody Production**

The production of polyclonal and monoclonal antibodies in animals has been critical for biomedical research progress for many years. This section will help you understand the ethical and procedural concerns that must be addressed when planning antibody production in animals and completing IACUC forms.

a. **Polyclonal Antibody Production**

In production of polyclonal antibodies, animals are typically immunized multiple times to elicit a strong antibody response, then bled so that immune serum can be collected and used in experiments. Two important considerations in producing polyclonal antibodies in animals are proper immunization technique and proper bleeding technique.

i. **Immunization Technique**
When producing polyclonal antibodies, adjuvants are usually mixed with antigens to augment the antibody response. The classic adjuvant is Freund's adjuvant, which is available in two forms:

- "Complete" (Complete Freund's Adjuvant, or "CFA"). CFA is a mixture of oils and water plus killed *Mycobacterium tuberculosis*. It typically elicits a very strong immune reaction. If used more than once, the immune reaction usually progresses to intense inflammation and sterile abscesses.
- "Incomplete" (Incomplete Freund's Adjuvant, or "IFA"). IFA is similar to CFA, but is missing the killed mycobacteria. This renders the IFA less effective as an immune stimulant, but it can be used safely multiple times without causing intense inflammation.

### ii. Use of Complete Freund's Adjuvant and Incomplete Freund's Adjuvant

To prevent inflammation and pain, CFA must only be used once. IFA is less inflammatory, and can be used multiple times. Typically, CFA mixed with antigen is administered to an animal the first time, then IFA is mixed with antigen for the second administration, then either IFA mixed with antigen or antigen alone is used for subsequent immunizations. The USDA states that the injection of CFA may cause more than momentary or slight pain. This means that CFA injections might necessitate assignment of USDA pain category D (painful/stressful but relieved) requiring the use of post-injection analgesics or sedatives.

### iii. Reducing Complete Freund's Adjuvant Side Effects

To reduce inflammation when using CFA, consider the following measures:

- Choose or make preparations of CFA with a lower mycobacterial concentration, i.e., 0.05 to 0.1 mg/ml, rather than 1 mg/ml.
- Add a concentrated antigen solution to the adjuvant to obtain a more antigen-rich emulsion, thereby reducing the volume of emulsion injected.
- Use multiple injection sites to limit the volume injected at any one site.
- Separate the injection sites to avoid fusion of inflammatory lesions.
- Maintain sterility of the antigen solution.

### iv. Complete Freund's Adjuvant and Incomplete Freund's Adjuvant Injections

The quantity of CFA or IFA adjuvant injected should be limited. Typical limits on adjuvant use are around 1 ml of combined adjuvant/antigen per immunization for rabbits (typically up to ten divided 0.1 ml injections), and around 0.25 to 0.5 ml combined adjuvant/antigen per immunization for smaller animals (up to ten divided 0.05 ml injections). These amounts have been shown to produce high titer antibodies, yet limit inflammation.

### v. Complete Freund's Adjuvant as a Health Hazard to Humans

If you are already sensitized to mycobacterial antigens by a previous exposure to CFA or through a natural infection of tuberculosis, you are likely to experience severe inflammation if you splash CFA into your eye.
or accidentally inject yourself with it. The inflammation and pain may be so severe that surgical removal of the site may be necessary. Protect your eyes and prevent accidental injection of yourself or a colleague when using CFA.

vi. Alternatives to Complete Freund’s Adjuvant and Incomplete Freund’s Adjuvant Injections
Less inflammatory alternatives to CFA and IFA are now available and in use. Examples are the block copolymer adjuvant Titermax®, and the lipid A-derivative adjuvant MPL® by RIBI. Other promising alternative adjuvants are also on the market. Such alternatives can be considered as a means of further reducing inflammation induced by Freund’s adjuvant.

vii. Choosing the Immunization Route
The route of immunization should be chosen to limit pain and inflammation. Regardless of the adjuvant used, the subcutaneous route typically provides a strong immune response, and is recommended. The intravenous route is not appropriate if adjuvant is used because the thick consistency of the adjuvant can result in lethal emboli in the blood stream. There are several other routes of immunization that are usually discouraged because there is little evidence that they offer any advantage over the subcutaneous route:

- **Intradermal (ID):** Causes more pain because the skin itself cannot stretch much as body fluids and white blood cells enter the immunization area, resulting in increased pressure and pain.
- **Intraperitoneal (IP):** Inflammation on surfaces of abdominal organs can result in peritonitis, granulomas, and pain.
- **Foot pad:** Injections can cause pain and lameness. When allowed by the IACUC, usually only one foot may be injected. Foot pad injections are usually discouraged in rodent species, and deemed inappropriate in larger species. Rabbits will often chew on their own feet after foot pad injections, presumably because of intense pain or irritation.

viii. Spacing Immunizations
Some evidence indicates that immunization injections should be spaced 3-6 weeks apart to elicit an optimal polyclonal antibody response, and the highest possible titer. There may be a temptation to hurry the process and shorten intervals, but a reduction in antibody titer may result. This is because circulating antibody from the previous immunization can remove antigen from circulation and thus limit its ability to induce a strong immune response.

ix. Blood Collection
Blood collection is obviously essential for collecting the immune sera from immunized animals. Often a "pre-bleed" is performed prior to immunization to determine if specific polyclonal antibody is already present in the animal (this could complicate some subsequent antibody studies). Periodic blood collections are needed thereafter to determine when a good antibody response is present. Once a good titer has been produced, serum per protocol objectives will be collected.
b. **Monoclonal Antibody Production**

NIH concurs with the findings and recommendations in the 1999 report of the National Research Council *Monoclonal Antibody Production* which indicates that during the accumulation of ascites there is likely to be pain and distress, particularly when some cell lines that are tissue-invasive are used and in situations of significant ascites development. The Report concluded that there is and will continue to be scientific necessity for this method, but that as tissue-culture systems are further developed, tissue-culture methods for the production of monoclonal antibodies should be adopted as the routine method unless there is a clear reason why they cannot be used.

Accordingly, IACUCs are expected to critically evaluate the proposed uses of the mouse ascites method. Prior to approval of such protocols, IACUCs must determine that (i) the proposed use is scientifically justified, (ii) methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, and (iii) the latter have been found unsuitable.

To produce monoclonal antibodies, animals (typically rodents) are immunized with an antigen, then spleen cells or lymph node cells are collected after euthanasia and fused with an immortal cell line. The fused cells are placed in a special medium that allows only hybrid cells to grow.

These hybrid cells, or hybridomas, are expanded in number, and the clones that produce antibody against the antigen of interest are saved.

If adjuvant is used during the immunization process, the same principles apply as described in the polyclonal antibody section.

i. **Two Uses of Animals in Generating Monoclonal Antibodies**

The first use of animals in generating monoclonal antibodies is to create the hybridoma cell line. The second common use of animals in generating monoclonal antibodies is to grow the hybridoma cell line on the peritoneal lining of histocompatible animals, and collect the antibody-rich ascites fluid.

ii. **Non-Animal Alternatives**

Over the past years, there have been a number of *in vitro* techniques introduced that can sometimes replace the use of animals for expanding hybridoma cell lines, and for collecting purified monoclonal antibody. Consequently, non-animal alternatives for generating purified monoclonal antibodies must be considered, and found to be unsuitable before the IACUC can approve animal use for that purpose.

iii. **Guidelines for Using Animals for Hybridoma Expansion**

When requesting approval to use animals for expanding hybridoma cell lines, be prepared to explain why *in vitro* techniques will not work. In 1999, The Committee on Methods of Producing Monoclonal Antibodies (sponsored by the Institute for Laboratory Animal Research and the National Research Council) suggested the following guidelines for IACUCs to use when evaluating the need for using animals for hybridoma expansion:
• When a supernatant of a dense hybridoma culture grown for 7–10 days (stationary batch method) yields a monoclonal antibody concentration of less than 5 mg/ml, or if other systems used yield concentrations less than 500 mg/ml (hollow fiber system) and 300 mg/ml (semi-permeable membrane system).

• When more than 5 mg of monoclonal antibody produced by each of five or more different hybridoma cell lines is needed simultaneously. It is technically difficult to produce this amount of monoclonal antibody because it requires more monitoring and processing capability than the average laboratory can achieve.

• When analysis of monoclonal antibody produced in tissue culture reveals that a desired antibody function is diminished or lost.

• When a hybridoma cell line grows and is productive only in the animal.

• When more than 50 mg of functional monoclonal antibody is needed, and previous poor performance of the cell line indicates that hollow-fiber reactors, small-volume membrane-based fermenters, or other techniques cannot meet this need during optimal growth and production.

These same criteria can help you decide if in vitro methods will suffice. The burden of proof is now on the investigator to show that in vitro methods of obtaining purified monoclonal antibody do not work, or are not effective in providing the amount of antibody needed.

iv. Guidelines for Using the Ascites Collection Technique

If in vivo methods are needed because in vitro methods cannot replace them, consideration must be given to minimizing the amount of pain and suffering involved. The following parameters should be considered when animals are used to expand hybridomas using the ascites collection technique:

• The amount of pristane used to “prime” the peritoneal cavity and make it better able to support hybridoma growth should be minimized (0.1 to 0.2 ml have been found to be effective).

• The degree of abdominal distension should be monitored at least daily and should distension begin to interfere with breathing, the ascites fluid should be removed.

• The number of peritoneal “taps” used to collect ascites fluid should be minimized. It is customary to limit withdrawals to two taps, unless the investigator provides evidence that the hybridoma is slow growing and additional taps can be accomplished in a humane fashion.

• The needle used should be as small as possible (20 gauge or higher). Because mice with ascites are not good anesthetic risks, ascites fluid is usually collected with a needle and syringe without anesthesia, and smaller bore needles cause less pain.
Endpoint criteria tailored to collecting ascites should be developed. Typical endpoint criteria include weight loss, extended anorexia, hunched posture, rough hair coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, or respiratory problems. Additional criteria to consider include a limit on the number of abdominal taps allowed, the presence of dyspnea (difficult breathing) unrelieved by a tap, and the development of solid hybridomas instead of more diffuse neoplasms producing ascites.
Principal Investigator (PI) submits protocol to IACUC by 1st business day of the month

Application screened for completeness by IACUC Office & either sent to PI for revisions or sent to Vet for Consultation

PI returns revision to IACUC

Vet Consult sent to PI by the 5th business day of the month

PI submits revised protocol by 10th business day of the month

Two presenters designated by the IACUC Chair

Presenters prepare summation of protocol, with any concerns, for the monthly meeting

Full Committee Review is done at the monthly meeting. A list of requested revisions is generated. IACUC discusses, evaluates, and votes to Approve, Reject or Return for revisions.

With signed agreement of all members present at the meeting, protocols requiring revisions are re-reviewed by designated member process unless a member(s) calls for Full Committee Review. Designated reviewers can approve the protocol, ask for additional revisions or ask for the protocol to be sent for full committee review.

If Returned for Revision, the IACUC sends requested revisions to PI by the end of the next business day following the IACUC meeting.

If Rejected the IACUC sends the reasons to the PI by the end of the next business day following the IACUC meeting.

If Approved the IACUC sends notification to PI by the end of the next business day following the IACUC meeting.

Designated Reviewers either agree to approve the protocol or send the protocol back to the PI with a request for further revisions.
Principal Investigator (PI) prepares amendment & submits to the IACUC

Amendment screened for completeness by IACUC Office & either sent to PI for revisions or sent to Vet for Consultation or sent to Vet & Chair for Administrative Approval

PI returns revised protocol to IACUC
Vet Consult sent to PI by the 5th business day
Administrative Approval sent to PI

PI submits revised protocol to the IACUC

Two designated reviewers appointed by the Chair. Amendment sent to IACUC to determine if Full Committee Review required (decision made in 2 business days) or if Designated Review is acceptable.

Designated Review done within three business days. A list of requested revisions is generated. Reviewer either Approve or Return for revisions.

If Returned for Revision, the IACUC sends requested revisions to PI by the end of the next business day.

Full Committee Review at monthly meeting

IACUC evaluates & votes on amendment to approve, request revisions or reject.

With signed agreement of all members present at the meeting, amendments requiring revisions are re-reviewed by designated member review process unless member(s) calls for Full Committee Review. Designated reviewers can approve the amendment, ask for additional revisions or ask full committee review.

Rejected:
IACUC sends the reasons to the PI by the end of the next business day

If Approved the IACUC sends notification to PI by the end of the next business day following the IACUC meeting

Designated Reviewers either agree to approve the protocol or send the protocol back to the PI with a request for further revisions.

PI submits revised protocol

Amendment Flow Chart Time Lines

If Returned for Revision, the IACUC sends requested revisions to PI by the end of the next business day.