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A. Georgia State University Institutional Animal Care and Use Committee (IACUC)

1. What Is The GSU IACUC?

The Institutional Animal Care & Use Committee (IACUC) is a standing committee at GSU 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, IACUC Assistant Director, principal investigators, laboratory staff, Research Occupational Health and Safety, and the Department of Animal Resources. The GSU IACUC uses the *Guide for the Care and Use of Laboratory Animals*, 8th Edition as its 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 Public Health Service (PHS) policy, and the Animal Welfare Act (Public Law 98-198), which states that the IACUC must consist of at least five members who are appointed by the institution’s chief executive officer (CEO). At GSU 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 GSU. 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

To share the responsibility as well as the workload, the GSU IACUC is composed of 13 voting members:

- 9 Scientists
- 1 Veterinarian
- 2 Community representatives
- 1 Nonscientist

The Attending Veterinarian (Director of the Division of Animal Resources) is a permanent appointment to the IACUC. The other members shall serve three-year rotating
appointments. Rotating 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.

The GSU IACUC utilizes 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 reviewed by the Institutional Official (IO).

2. **IACUC Oversight**

As a decision making body of GSU, the IACUC ensures that all animals in experimental research 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 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.

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 proposal. 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) relevant to these procedures must be indicated. If such experience is lacking, the investigator must indicate how they 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 subcontractors, 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 activating an individual’s Panther Card 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.

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 GSU animal users.

   For the initial sign up, click on “Enroll Now” on the AALAS Learning Library home page. Then select “Join A Group”, and use the access code "traininggsu" (not case sensitive) to set up a personal Log-In ID and Password.

3. **Required Modules**

   Required training modules are dependent upon the species with which one works as well as the procedures which one performs. The required species- and procedure-specific training can be found on the GSU Institutional Animal Care and Use website [http://www.gsu.edu/research/iacuc.html](http://www.gsu.edu/research/iacuc.html) under the link entitled “Required Education.”

4. **Module Completion**

   The IACUC is automatically notified of the individuals 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, you can contact the GSU IACUC Assistant Director 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 GSU IACUC Assistant Director 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.

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 the approval form is signed by both the IACUC Chair and the Principal Investigator, the individual will be added to the protocol and an approval letter will be sent to the principal investigator.  
   [http://www.gsu.edu/images/vp_research/GSU_IACUC_Personnel_Amendment_Form.doc](http://www.gsu.edu/images/vp_research/GSU_IACUC_Personnel_Amendment_Form.doc)

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 are 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 GSU 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 GSU 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 GSU. 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.

10) I program software to for use in testing animals in my approved research protocol at GSU. If I provide my software to other researchers to use, do they need complete the training?

No. They do not need to complete the training.

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

All animal users must enroll in the Research Occupational Health and Safety Program (ROHSP). To do so, it is necessary to read the Medical Monitoring Program for Vertebrate Animal Exposure (MMPVAE) informational document and complete the MMPVAE Enrollment Form. Both are found on the ROHSP web page (http://www.gsu.edu/research/rohs.html). Follow the directions on the form for submission. Please note that enrollment in the program (completion of the MMPVAE Enrollment Form) is mandatory. One is entitled, however, to decline recommended services by signing the waiver form indicating such.

D. Proposal 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.

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).

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.

Additionally, occupational health and safety issues, including zoonosis, should be reviewed by the institution’s health and safety committee or office, with assurances to the IACUC that the field study does not compromise the health and safety of either animals or persons in the field. Principal investigators conducting field research should be knowledgeable
about relevant zoonotic diseases, associated safety issues, and any laws or regulations that apply. Exceptions to the above should be clearly defined and evaluated by the IACUC.

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.

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 to ensure that no significant deviations from established and approved procedures have occurred. All 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 should be 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

• method 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

Prior to the 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.

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.

Generally, the FCR method will be used. 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.

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.

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

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

b. 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.

c. 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.

d. 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.

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

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

g. 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.

IACUC Member participation is facilitated through the IACUC Compliance Office. Please contact the IACUC Assistant Director at (404) 413-3508 for more information.

1. Submission and Processing of Animal Use Applications

All Protocol Submission and Amendment Forms can be found on the IACUC page of the Georgia State website: [http://www.gsu.edu/research/iacuc.html](http://www.gsu.edu/research/iacuc.html)

2. IACUC Meetings

IACUC meetings are held on a monthly basis. See the online IACUC calendar for specific meeting dates and deadlines ([http://www.gsu.edu/research/iacuc_calendar.html](http://www.gsu.edu/research/iacuc_calendar.html))
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 GSU’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 Care Office.

4. New Protocol Submission

Principal investigators are required to complete and submit an “Application for a New Protocol” 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 Occupational Health and Safety 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 Occupational Health and Safety Program can be found on the Research Occupational Health and Safety web site http://www.gsu.edu/research/rohs.html

Consultation with the Attending 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 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. 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 by the IACUC Chair prior to new personnel 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 the Attending 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. At this time
the veterinarian will review the protocol amendment form and provide written comments so that revisions to the form, if suggested, can be made prior to IACUC review.

**Significant vs. Minor Changes**

ALL changes in ongoing active protocols must be submitted to the IACUC using the Protocol Amendment form. The IACUC Chair and/or Attending 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. Examples of changes considered to be significant include, but are not limited to, changes:

a. in the objectives of a study
b. from non-survival to survival surgery;
c. resulting in greater discomfort or in a greater degree of invasiveness;
d. in the species or in approximate number of animals used\(^1\);
e. in Principal Investigator;
f. in anesthetic agent(s) or the use or withholding of analgesics;
g. in the method of euthanasia; and
h. in the duration, frequency, or number of procedures performed on an animal

Protocol amendments with changes that are not deemed “significant” based on a thorough evaluation by the IACUC Assistant Director, in coordination with the IACUC Chair and/or Attending Veterinarian can be approved administratively.

**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).

GMAs, particularly mice and fish, are important animal models, and new methods and combinations of genetic manipulation are constantly being developed (Gondo 2008). Regardless of whether genetic manipulation is targeted or random, the phenotype that initially results is often unpredictable and may lead to expected or unexpected outcomes that affect the animal’s well-being or survival at any stage of life. For example, in some instances genetic modification has led to unforeseen immunodeficiency, requiring the GMA offspring to be held under specialized bio-exclusion conditions (Mumphrey et al. 2007); and the promoter

\(^1\) 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).
sequences used to direct expression of transgenes to specific tissues have varying degrees of specificity (“leakiness”) that can lead to unanticipated phenotypes (Moorehead et al. 2003). These examples illustrate the diversity of unanticipated outcomes and emphasize the need for diligent monitoring and professional judgment to ensure the animals’ well-being (Dennis 2000). The first offspring of a newly generated GMA line should be carefully observed from birth into early adulthood for signs of disease, pain, or distress. Investigators may find that the phenotype precludes breeding of particular genotypes or that unexpected infertility occurs, situations that could lead to increases in the numbers of animals used and revision of the animal use protocol. When the initial characterization of a GMA reveals a condition that negatively affects animal well-being, this should be reported to the IACUC, and more extensive analysis may be required to better define the phenotype (Brown et al. 2000; Crawley 1999; Dennis 2000). Such monitoring and reporting may help to determine whether proactive measures can circumvent or alleviate the impact of the genetic modification on the animal’s well-being and to establish humane endpoints specific to the GMA line.

When unforeseen animal welfare concerns arise during the conduct of an IACUC approved animal procedure, the University Veterinarian must be consulted immediately. The University Veterinarian will work with the Principal Investigator to find a solution that addresses the concern. The University 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 University 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 GSU IACUC Protocol Amendment Form to the IACUC office within five (5) business days after receipt of the University 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).

6. Notification of Protocol Status

The IACUC Office sends the PI notice, in writing, 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 file is updated to note the date of approval and a letter of approval along with the approved protocol and/or amendment is sent to the investigator. Copies of the approvals are also kept in the IACUC Office.
If the protocol requires modification

The investigator is informed of the modifications needed and returns a response. The response is sent to the reviewers who have four (4) 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, the IACUC Chair signs the approval letter.

If further detail is necessary, the investigator is notified and returns a response. If the response is acceptable to all reviewers the IACUC Chair signs the approval letter.

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 by the IACUC.

7. Post-Approval Monitoring

The DAR management staff (Director, Associate Director, Assistant Director, Supervisor, and Animal Healthcare Technician) conducts regular animal facility rounds to include an assessment of medical record keeping, adherence to humane endpoint criteria, animal enrichment provisions, congruence between approved animal procedures and actual animal procedures, etc. In addition, the DAR laboratory animal technicians serve a critical role in this process as they are in the animal facilities on a daily basis. Concerted efforts are made to work closely and collegially with the research faculty and staff with the intent of promoting an environment of compliance and quality animal research and care. Further, the Assistant Director, IACUC strives to conduct extensive post approval monitoring on approximately 10% of the IACUC-approved protocols yearly.

The goal of Post Approval Monitoring is to work with, and in support of, the researcher, research staff member, and students to confirm that accurate and consistent protocol performance is pursued in a collegial and unobtrusive manner. Post-approval Monitoring by the Institutional Animal Care and Use Committee (IACUC) of protocols is performed to provide assurance to regulatory agencies and the Georgia State University IACUC that animal experiments are performed in accordance with approved IACUC protocols. Investigators and laboratory staff will work with the Assistant Director, IACUC to observe and confirm monitoring procedures with the approved protocol. The Assistant Director, IACUC will work with the investigator and laboratory staff to observe research activity. Afterword accurate reports are
prepared, recommendations for maintaining compliance is provided, and PIs are informed of training opportunities.

The Assistant Director, IACUC shall use a checklist for the routine post approval monitoring.

During each post approval monitoring session, the Assistant Director, IACUC 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.

Such 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 Assistant Director, IACUC 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 Assistant Director, IACUC 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 the Attending Veterinarian for immediate resolution.

The Assistant Director, IACUC 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 Assistant Director, IACUC shall send a final written report of the monitoring results to the Principal Investigator, IACUC Chair and Attending 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 Assistant Director, IACUC will follow up on any issues that require protocol modifications, orientation of new personnel, or training. The Assistant Director, IACUC 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 protocol file.

The Assistant Director, IACUC 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.

8. Three Year Review of Protocols

Protocols are approved for three years by the Institutional Animal Care and Use Committee (IACUC) based on Public Health Service (PHS) Policy guidelines and subject to annual review through the IACUC Protocol Continuation or Cancellation form. The IACUC will conduct a continuing review of each previously approved, ongoing activity (sponsored or non-sponsored) covered by PHS Policy at appropriate intervals as determined by the IACUC, including a complete review in accordance with the PHS Policy at IV.C.1-4, at least once every three years. Protocol compliance and review of ongoing protocols or modification of approved protocols will be handled by: (1) requiring that principal investigators submit Progress Report to the IACUC indicating the status of their research on an annual basis; (2) when placing animal orders the Animal Health Technician verifies that the approved number of animals has not been exceeded; and (3) the Attending Veterinarian and/or IACUC Assistant Director, whenever possible, observes animal research procedures in progress.
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 it does not appear that a renewal protocol 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. For the non-traditional research animals such as fish, lizards, etc., the PI will continue to provide the specialized husbandry tasks. These animals will be maintained by the PI, but not otherwise utilized until a new protocol has been submitted. Letter “d” below will be strictly adhered to.
      ii. All husbandry activities will be performed by Division of Animal Resources staff at PI expense, regardless of where the animals are housed.
      iii. While on the holding protocol, no breeding, handling or work with the animals may be performed.
   b. If the PI is currently breeding animals on the expired protocol, Division of Animal resources will separate all breeding pairs, also at the PIs expense.
   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 is not submitted prior to the expiration date, all animals will be euthanized within 30 days of the animals moving to the holding protocol.

Upon approval of the new protocol, the animals are transferred from the holding protocol to the new protocol at PI expense. Research activities may commence at this time
9. **Suspension of Research Activity**

The 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.

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 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.
10. **Protocol Flow Chart**

Principal Investigator (PI) submits protocol to IACUC by 1st business day of month

Application screened for completeness by IACUC Compliance Officer and sent to the University Veterinarian for consultation.

Veterinary Consultation sent to PI by the 5\textsuperscript{th} business day of the month

PI submits revised protocol by 10\textsuperscript{th} business day of month

Protocol & Vet Consult sent to IACUC members. Two presenters designated by IACUC Chair.

Presenters prepare summation of protocol with any concerns on the Protocol Review Form for the monthly meeting.

Full Committee Review at monthly meeting. List of requested revisions generated. IACUC discusses, evaluates, and votes to Approve, Reject, or Return protocol for revision.

If Returned for Revision the IACUC Compliance Officer sends requested revisions to PI via email by the end of the next business day following the IACUC Meeting

If Approved the IACUC Compliance Officer sends notification to PI via email by the end of the next business day following the approval.

If Rejected the IACUC Compliance Officer sends the reasons to PI via email by the end of the next business day following the IACUC Meeting

With the agreement of all of the members present at the meeting, protocols requiring scientific revisions will be re-reviewed by the designated review process unless one of the committee members not attending the meeting calls for a full committee review of the revised protocol within one business day of receiving the Committee’s comments. The designated reviewers can approve the revised protocol, ask for additional modifications by the PI or ask that the revised protocol be sent to the full committee for review.

If the committee concerns are non-scientific, the revised protocol is reviewed by the IACUC Compliance Officer or IACUC Chair

PI submits revised protocol
11. Amendment Flow Chart

Principal Investigator (PI) prepares amendment & submits to IACUC office

Amendment screened for completeness by IACUC Compliance Officer

Amendment sent to Attending Veterinarian for consultation

IACUC Compliance Officer returns to PI for revision if needed

Minor change: Approval sent to PI

PI re-submits revised amendment to the IACUC Compliance Officer

Amendment sent to IACUC to determine if Full Committee Review is required or if Designated Review is acceptable.

Decision made in 2 business days

Full Committee Review at monthly meeting

Designated reviewers evaluate amendment within 3 business days

Send for Full Committee Review

IACUC evaluates and votes on amendment

Approve, return to PI for revision, or reject (by full committee only)
E. Completing the IACUC Protocol Form

1. Criteria for Granting Approval of Protocols (per Animal Welfare Act Mandates and the Guides)

   • 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. Attending 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 several 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 qualified.

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

2. **Explaining 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 might result 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 Your 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 individual animals. 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. **Selecting the Species**

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.

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 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, 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, 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 your Attending 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. Requirements and Search Procedures for the Identification and Evaluation of Alternatives to Animal Use

In accordance with the Health Research Extension Act of 1985, scientists performing painful 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
• INSIDE CONFERENCES
• LABORATORY ANIMAL LITERATURE circa 1920s to the present
• NORINA (Norwegian Inventory of Audiovisuals)
• SERLINE
• STRAIN DESCRIPTION

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/. 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.

**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 sequelae 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.

The criterion used for intervention in research studies to prevent unnecessary pain and distress is called "endpoint criteria" because they describe when it is time to either:

- Euthanize an animal to prevent suffering;
- Discontinue a painful procedure; or
- Remove an animal from a study.

Common examples of endpoint criteria include a limit on weight loss as a percentage of body weight (but body weight must be monitored throughout); anorexia for an extended time (as monitored by measuring food intake); sudden pain or distress that cannot be controlled with analgesics, sedatives or tranquilizers; or severe medical conditions that cannot be controlled with appropriate therapy (e.g. severe systemic infections, kidney or liver failure, heart disease).

More specific criteria are often used for certain types of studies. For example, endpoint criteria used for rodent cancer studies involving the growth of tumors under the skin often include:

- 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.

Endpoint criteria for mice used to produce ascites fluid rich in monoclonal antibodies will be discussed in Section 14 below.

Humane endpoint criteria should be addressed on the IACUC protocol form when it is anticipated that an animal will endure painful or distressful conditions.

**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 Attending 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.

Guidelines on morbundancy and times for intervention by euthanasia include: Impaired ambulation (unable to reach food or water easily, inability to remain upright); evidence of
muscle atrophy or other signs of emaciation; any obvious prolonged illness including such signs as lethargy (drowsiness, aversion to activity, and/or a lack of physical or mental alertness), prolonged inappetence; difficulty breathing; central nervous system disturbances; or chronic diarrhea or constipation

12. 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; reversibility; 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.

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

Concerns or complaints regarding animal usage at GSU 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](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, the Attending 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 GSU 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. GSU 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. Additional Considerations Pertaining to Protocols

1. Anesthetic Machine Maintenance

As a component of maintaining properly functioning anesthetic machines, such machines should receive an inspection on a regular basis by a qualified service technician. Such an individual is able to check for leaks, replace worn parts, and check the calibration of the vaporizer. 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. Should the vaporizer be in need of calibration then it can be replaced with a “loaner” vaporizer while it is removed from the premises for calibration. Such an inspection is often conducted on a biennial basis.

GSU IACUC has established a standard operating procedure requiring vaporizers to be inspected on a biennial basis. As the service technician operates on an hourly basis (vs. a per machine charge) then it is most economical to schedule the technician to come on a given day and allow this individual access to all machines on this day. The bill will then be divided up per machine and billed to the labs that own the machines. The Animal Resources Animal Healthcare Technician will be responsible for contacting the research laboratories having anesthetic machines to coordinate the visit by the service technician.

Activated charcoal scavengers (e.g. F/Air canisters) are only able to adsorb a certain amount of anesthetic gas. Scavengers must be weighed prior to being put into use and the starting weight recorded

Scavengers 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).

The frequency of weighing the scavengers depends on the frequency and duration of use.

It is suggested that the canisters are weighed after each day of use, or empirically every month.

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 in regular trash.
2. Animal Transportation

Animal Transport on the GSU Campus

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 (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:

1) NSC: Empty cages should be placed on the provided cart in the foyer of the animal facility.

2) 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.

3) NSC and PSC: Occupied cage exteriors should be sprayed down with 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.

4) 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.

3. Animals on Expired Protocols

All animals maintained in the GSU animal research facilities must be held under a current, valid, IACUC-approved protocol. In addition, no animal activities except for medical care prescribed by a veterinarian may be carried out without an IACUC-approved protocol.

Accordingly, the Department of Animal Resources (DAR) has an IACUC-approved Holding Protocol to maintain surplus animals until their disposition can be determined. Surplus animals may include extra animals born but not used in research, animals remaining after use on an IACUC-approved protocol, surplus animals received in an animal shipment, animals which
reside on a protocol which expires and are in need of being transferred to an IACUC-approved protocol for "holding" until the new protocol has been reviewed and approved by the IACUC.

Many of these surplus animals will eventually be transferred to another IACUC-approved protocol. Some of these animals will be adopted as pets, transferred to the zoo if the zoo has a need for such an animal, or euthanized. Some of the rodents that are euthanized via carbon dioxide may be donated as a food source (e.g. to a wildlife rehabilitation center).

Any animal adoptions will involve the use of a liability form that was developed and approved with the input of GSU legal counsel. In addition, any animals that are transferred between protocols will involve the use of the Animal Transfer Form to ensure appropriate oversight and approval is in place to include the prevention of the overuse of a particular animal.

4. Surgery

Surgery on animals requires highly trained, conscientious individuals, and appropriate prior planning. To understand the issues involved, some important terms and concepts must be addressed. Surgery is addressed in some detail in the animal protocol forms.

a. 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.

b. General Anesthesia

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

c. 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.

d. Survival Surgery

It is important to distinguish between survival and non-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.
e. **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.

g. **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 the attending 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.

h. 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.

i. 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 the Attending Veterinarian during the planning stages of the experiments.

**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.

**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.

**j. 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.**

**5. 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](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.

c. **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.

d. **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:

- **Exsanguination** - due to anxiety associated with low blood pressure.
- **Decompression** - numerous disadvantages.
- **Rapid freezing** - not humane.
- **Air embolism** - the injection of air intravenously, can be accompanied by convulsions, other neurological signs.
- **Drowning** - not humane.
- **Strychnine** - causes violent convulsions and muscle contractions.
- **Nicotine, magnesium sulfate, potassium chloride** - cause cardiac and/or respiratory arrest before unconsciousness.
- **Chloroform** - known toxicity for animals and humans.
- **Cyanide** - extreme danger to humans and other animals.
- **Stunning by a blow to the head** - may not cause death and aesthetically objectionable.
e. **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.

f. **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.

g. **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.

**h. 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.

**i. 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.

j. **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.

k. **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.

6. **Use of Hazardous and Toxic Agents**

a. **Using Hazardous and Toxic Agents in Animals**

The GSU 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.

b. **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.

c. **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.

d. **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.

e. **Recombinant DNA**

There are additional guidelines to consider if your work in animals includes:

http://oba.od.nih.gov/oba/rac/guidelines/ni_h_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.

f. 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/bmb15/

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 that are 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 that are 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 that are 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 that are 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

g. Explosive Agents in the Animal Facility

In the GSU 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.

7. Weight Loss as an Endpoint

Immature animals: maximum weight loss is a deviation of 15% 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.

8. 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.

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 Research Animal Diagnostic and Investigative Laboratory (RADIL) at the University of Missouri offer 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. Typically, IMPACT testing requires 2 vials of each sample with a minimum of $1 \times 10^7$ 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 the Attending Veterinarian the name of the cell line(s), source, test, and results of tests performed to evaluate the presence of rodent pathogenic microorganisms. 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 the Attending Veterinarian to make arrangements to have biological specimens tested before use. Approval for the use of biological materials in animals housed at GSU will only be given after the Attending Veterinarian has assessed the test results to determine their adequacy.

9. **Food or Water Restriction**

Regulation of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioral research protocols. The regulation process may entail scheduled access to food or fluid sources, so an animal consumes as much as desired at regular intervals, or restriction, in which the total volume of food or fluid that is consumed is strictly monitored and controlled. The objective when these studies are being planned and executed should be to use the least restriction necessary to achieve the scientific objective while maintaining animal well-being.

The development of animal protocols that involve the use of food or fluid regulation requires the evaluation of three factors: the necessary level of regulation, potential adverse consequences of regulation, and methods for assessing the health and well-being of the animals. In addition, the following factors influence the amount of food or fluid restriction that can be safely used in a specific protocol: the species, strain, or stock, gender, and age of the animals; thermoregulatory demand; type of housing; time of feeding, nutritive value, and fiber content of the diet (Heiderstadt et al. 2000; Rowland 2007); and prior experimental manipulation. The degree of food or fluid restriction necessary for consistent behavioral performance is influenced by the difficulty of the task, the individual animal, the motivation required of the animal, and the effectiveness of animal training for a specific protocol-related task.

The animals should be closely monitored to ensure that food and fluid intake meets their nutritional needs (Toth and Gardiner 2000). Body weights should be recorded at least weekly and more often for animals requiring greater restrictions. Written records should be maintained for each animal to document daily food and fluid consumption, hydration status,
and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol. In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended.

Restricted access to food or water must be tailored to the species under study. When beginning work with a new species, consult with the Attending Veterinarian as well as the literature when designing and describing protocols for fluid or food restriction.

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, the cage must be labeled with the date and time the deprivation will start, the date and time the deprivation will end, the name and telephone number of the individual responsible for replacing the food and/or water, and a statement such as “WITHHOLD FOOD AND WATER” or “WITHHOLD WATER.”

10. **Blood Collection**

During blood collection, the volume and frequency of collection must be carefully limited so that neither shock nor anemia results.

How much blood can be collected at one time? One simple guideline is to collect no more than 1% of the body weight at one time. Assume that a ml of blood weighs 1 gram. For example, for a 4,000 gram rabbit, 40 ml (0.01 x 4,000= 40 ml) could be safely collected. For a 20 gram mouse, 0.2 ml (0.01 x 20= 0.2 ml) could be safely collected.

How often can blood be collected? In general, multiple blood collections should be spaced far enough apart to prevent anemia and distress in animals. Blood collections of no more than 1% of body weight can usually be performed every 2 weeks without harm. More frequent collections might require fluid replacement and/or hematocrit monitoring to prevent severe anemia in an animal.
Blood collection from the heart should only be performed on anesthetized animals as a terminal procedure.

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

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. Other expired materials should not be used unless the manufacturer verifies efficacy beyond the expiration date, or the investigator is able to document to the satisfaction of the IACUC that such use would not negatively impact animal welfare or compromise the validity of the study. The veterinarian and IACUC must maintain control over the use of expired medical materials in order to meet their responsibilities to avoid or minimize discomfort, pain or distress to animals.

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.

12. **Use of Non-Pharmaceutical-Grade Chemicals**

The use of pharmaceutical-grade chemicals and other substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals. They should therefore be used, when available, for all animal-related procedures. The use of non-pharmaceutical-grade chemicals or substances should be described and justified in the animal use protocol and be approved by the IACUC (Wolff et al. 2003); for example, the use of a non-pharmaceutical-grade chemical or substance may be necessary to meet the scientific goals of a project or when a veterinary or human pharmaceutical-grade product is unavailable. In such instances, consideration should be given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use.

13. **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.

14. Prolonged 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.
15. 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 CFA and IFA

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.

Reducing CFA 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.

### iii. CFA and IFA 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.

### iv. CFA 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!

### v. Alternatives to CFA and IFA

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.

### vi. 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.

vii. **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.

viii. **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 histo-compatible 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 fermentors, 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.

**16. 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.”

Accordingly, all animal acquisitions must be made via the GSU 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.

17. Guidelines for Approving Animal Transfers between IACUC-Approved Protocols

The Division of Animal Resources Director or Senior Administrative Coordinator in consultation with the IACUC Compliance Specialist 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.

18. Animal Stabilization Procedure Pertaining to Newly Acquired Animals

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 are 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.

19. Animal Medical, Surgical and Research Records

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 Laboratory Animal Care Record (LACR). 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 University 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 LACR will be associated with each IACUC protocol represented in the animal housing room. 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://www.gsu.edu/research/43247.html). Should you observe an animal health issue, please 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 Technician (office: 404-413-3594; mobile: 404-709-9910; e-mail: cbillinglssey2@gsu.edu) or the Attending Veterinarian (office: 404-413-3553; mobile: 404-391-7366; e-mail: mhart@gsu.edu). In the event of an emergency please contact these 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://www.gsu.edu/research/43247.html). 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. Examples 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 cards which contain the biohazard symbol and a place to record the name of the infectious agent and the date it was administered to the animal
7. Regarding the administration of substances in the water, DAR will provide cage cards which contain a place to record the substance which has been added to the water
8. Regarding breeding rodents, DAR will provide cage cards which contain places to record relevant breeding information

20. **Mouse Breeding Trios**

GSU 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 Procedure 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.”

21. **Social Housing of Social Species**

Social housing of social species will be considered by the GSU IACUC 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.
22. **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 a 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 and can be found at [http://www.gsu.edu/images/vp_research/animal_enrichment_plan.doc](http://www.gsu.edu/images/vp_research/animal_enrichment_plan.doc)

23. **Mouse Tumor Burden**

Guidelines have been developed that will 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 determine 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. These guidelines can be found at: [http://www.gsu.edu/images/vp_research/GSU_IACUC_Policy_on_Mouse_Tumor_Burden.doc](http://www.gsu.edu/images/vp_research/GSU_IACUC_Policy_on_Mouse_Tumor_Burden.doc)

24. **Approved Housing Areas**

The following locations at GSU have been approved by the IACUC for housing animals longer than 12 hours:

- Language Research Center, Animal Facility
- Natural Science Center, basement, Animal Facility
- Natural Science Center, 5th floor, Aquatic Housing Room
- Petit Science Center, 8th floor, Aquatic, Reptile and Amphibian Housing Rooms
- Petit Science Center, 9th floor, Animal Facility
- SunTrust Building, 1st floor, Animal Housing Rooms