TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER™

Institutional Animal Care & Use Committee

POLICIES

Revised December 12, 2025

in concurrence with TTUHSC PHS Assurance D16-00032, and other Federal Regulations and Guidelines

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Introduction

These Institutional Animal Care and Use Committee ("IACUC") Policies apply to all animal research, teaching, and testing on all TTUHSC campuses and are carried out under the authority of TTUHSC.

General

TTUHSC is subject to applicable state and federal laws and regulations that include but are not limited to the Animal Welfare Act and federal regulations implementing the Animal Welfare Act; the Health Research Extension Act of 1985; the Public Health Services Policy on the Humane Care and Use of Laboratory Animals; and the provisions and principles set forth in the most recent editions of the Guide for Care and Use of Laboratory Animals, published by the National Academy of Sciences, and the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching published by the Federation of Animal Science Societies.

TTUHSC Operating Policies

TTUHSC OP 73.03, Animal Care and Use Program describes the administration, responsibilities, and authority that form the framework for the use of animals in research, teaching, and testing at TTUHSC.

The Institutional Animal Care and Use Committee (IACUC) is the committee established by the Institutional Official (IO) for the TTUHSC President for assessment and oversight of the Animal Care and Use Program, its components, and facilities. The IACUC reviews and approves animal research and teaching protocols, inspects animal research facilities, and provides feedback to the IO to support and to protect the approved use of animals in research, teaching, and testing at TTUHSC. The Chair of the IACUC and other committee members will be appointed by the IO.

The Institutional Official (IO), designated by the TTUHSC President, is the Senior Vice President for Research and Innovation (SVPRI).

Applicability

The IACUC, with oversight by the Institutional Official, hereby establishes policies and procedures governing animal research at TTUHSC. These policies apply regardless of whether the activity is subject to Federal regulation, with whom it is conducted, or the source of funding support (i.e., sponsorship).

All research, teaching, and testing involving animals will be governed by these policies and procedures if the research or teaching is conducted by or under the direction of any employee or agent of TTUHSC using any TTUHSC property or facility.

Policy Non-Compliance

Failure to comply with the TTUHSC IACUC Policies and Procedures may result in remedial action by the IACUC including, but not limited to, revoking or terminating approved IACUC protocol(s), requiring remedial training, reporting non-compliance to Department Chairs or other Senior Administration, and/or to Federal Funding Agencies. Any perceived violation of IACUC Policy must be reported in accordance with IACUC Policy #10, even if immediate harm to animals is not present.

Confidential Medical Committee

The IACUC, and any sub-committees established under these Policies and Procedures, shall each be considered a “medical committee” as defined under Texas Health & Safety Code §161.031, and/or other applicable state and federal statutes. All documents generated by the IACUC, submitted to the IACUC, or created for the purposes of fulfilling IACUC duties are confidential and privileged and shall be identified as a "Confidential - Medical Committee Document." Persons with access to IACUC documents shall take adequate steps so that such information is not used by or made accessible or released to unauthorized sources.

Records Retention

All IACUC records shall be maintained for a minimum of three (3) years.

However, records that are related directly to applications, proposals, and proposed significant changes in ongoing activities reviewed and approved by the IACUC shall be maintained for the duration of the activity plus a minimum of an additional three (3) years after completion of the activity. See the PHS Policy on Humane Care and Use of Laboratory Animals and the USDA Animal Welfare Act.

Policy Creation and Review

All potential new policies will be approved or rejected by a simple majority of the voting members during a regularly scheduled IACUC meeting.

All published IACUC policies will be reviewed by the IACUC periodically or as the need arises in order to:

- continue the policy without change;
- revise the policy; or,
- rescind the policy.

The goal will be to review each policy at least once every three years.

Inquiries about TTUHSC Animal Care and Usage

Internal inquiries regarding confirmation of protocol approval or status of research or teaching at TTUHSC may be directed to the IACUC Administrator in the Research Integrity Office (RIO). Only inquiries from an approved member of the study team, the Department Chair or Dean of a member of the study team, or representatives from TTUHSC Compliance or TTU System Audit Services or Office of General Counsel may be answered by the IACUC Administrator. All other requests for information will be treated as public requests for information, as described below.

All media or other external inquiries (letter, telephone, personal visit, etc.) and any presentation (video, oral or written) regarding TTUHSC Animal Care and Usage shall be referred to the IACUC Administrator in the Research Integrity Office. Such requests or presentations will require review and approval from an ad hoc committee comprised of some or all of the following, depending upon the nature of the request:

- the SVPR/Institutional Official or designee
- the IACUC Chairperson or designee
- the IACUC Administrator or designee from the Research Integrity Office
- the Institutional Veterinarian
- a representative from the TTU System Office of General Counsel
- a representative from TTUHSC Communications and Marketing.

Conflicts of Interest

Should there be a Conflict of Interest or the appearance of a possible Conflict of Interest, defined per HSC OP 10.05, concerning the execution of any IACUC Policy or Procedure, then the matter will be forwarded to the IACUC Chair for resolution. Should the Conflict of Interest, or appearance of possible Conflict of Interest, involve the IACUC Chair, then the matter will be forwarded to the IACUC Vice-Chair for resolution. Should the Conflict subject matter involve both the IACUC Chair and Vice-Chair, the matter will be forwarded to the Institutional Official (IO) for resolution.

HSC OP 10.05 defines Conflict of Interest as a situation in which an Employee's financial, professional, or other personal considerations may directly or indirectly affect, or have the appearance of affecting, the Employee's judgment in exercising any duty or responsibility including the conduct or reporting of research owed to the Institution.

Policy 1: Animal Use Performed by/for TTUHSC Investigators at Non-TTUHSC Sites

The IACUC recognizes that TTUHSC investigators frequently collaborate on animal research with investigators from other institutions and that animals involved in such research will be housed at the collaborator's institution. To ensure that this research conforms to the standards of the TTUHSC, the following policy has been formulated.

1. If a TTUHSC investigator will be involved in animal research (a) where animals will be housed and/or research will be conducted at a non-TTUHSC site, institution (for example custom-made animals or antibodies), or non-affiliated business (for example, a privately-owned farm), or (b) animals will be purchased with TTUHSC funds regardless of the site, the TTUHSC investigator must supply the following information to the IACUC administrator:
   a. The name of the PI at TTUHSC
   b. The name of the PI or responsible individual at the other institution, agency, or business
   c. The name of the collaborating institution, agency, or business
   d. IACUC title and protocol number assigned by the other institution, agency, or business, if applicable
   e. Species involved in the IACUC protocol
   f. The date of the current IACUC approval for this protocol, and the period it covers (i.e., 5/11/24 - 5/11/25)
   g. PHS Assurance number[1,2]
   h. USDA Registration number (if USDA-covered species)
   i. Written confirmation of current IACUC approval from an authorized official at the other site, if applicable
   j. Brief statement of what will be done at or provided by the non-TTUHSC site.

2. If a TTUHSC investigator will be purchasing custom-made animals or antibodies from a commercial source, the TTUHSC investigator must supply the company's PHS Assurance number.

3. If a TTUHSC investigator is on sabbatical leave at a non-TTUHSC institution, the TTUHSC investigator must abide by the policies, rules and regulations of the designated sabbatical institution when using animals at that institution. It is understood that in this instance, the TTUHSC IACUC is not responsible for the investigator's activities at the sabbatical institution during the investigator's leave. However, the use of TTUHSC funds supporting any animal investigation at the sabbatical institution also must conform to the policy defined in item 1.

References

1. Public Health Service Policy on Humane Care and Use of Laboratory Animals. V.B.
2. Silverman, J., Suckow, M.A. & Murthy, S. (2014). *The IACUC Handbook*, 3rd ed. p.166

Policy 2: Review of Protocols and Housing of Animals between TTU and TTUHSC

Pursuant to the Memorandum of Understanding approved on January 21, 2022, by Texas Tech University (TTU) and Texas Tech University Health Sciences Center (TTUHSC), each agrees to the following:

1. To abide by the Animal Welfare Act.

2. To have an active Letter of Assurance with the Public Health Service for animal care and use.

3. To constitute a functional IACUC.

4. That the use of vertebrate animals in testing, research, and teaching must be reviewed and approved by the IACUC at the institution that will house the animals.

5. That the principal investigator (PI) shall house his/her animals within his/her home institution (where he/she has their primary appointment) unless the PI identifies "special needs" that can be better served at the other institution. The attending veterinarian at the PI's home institution must verify such "Special needs" in writing. This verification (that the PI has been granted permission to submit a protocol to the other institution) is to be included with the submission of the protocol. "Special needs" could include:

   A. The inability of the home institution to house the animals.

   B. Collaboration between the PI of the protocol and an investigator at the other institution.

   C. Special facilities required by the project that are unavailable at the PI's home institution.

6. That all personnel involved with the project must meet the training and occupational health requirements of the institution where the animals are housed.

7. That the respective IACUCs will send each other copies of approved protocols that meet the criteria in number five above.

8. That this policy supersedes any previous agreements/policies/understandings between the institutions regarding the review of protocols and housing of the animals.

Policy 3: Procurement, Housing & Accountability

1. Procurement

The following procedure must be followed regardless of the funding source used for procurement.

A. Live vertebrate animals ("live animals") used for teaching or research, under the auspices of TTUHSC, may be ordered only if an approved IACUC protocol exists for that purpose.

B. All orders for live animals must be processed through the appropriate TTUHSC LARC.

C. All live animals ordered must be delivered to the appropriate TTUHSC LARC.

D. Upon arrival the animals must be checked by the LARC for the correctness of the order and for the animals' health status. The LARC will notify the principal investigator (PI) of the animals' status, and the animals will be housed in the LARC.

E. The live animals ordered by the LARC or transferred onto a protocol must not exceed the number approved under the IACUC protocol.

2. Housing

A. All animals must be housed within the appropriate TTUHSC LARC except when specified in the approved IACUC protocol.

B. USDA-regulated animals (which include all warm-blooded vertebrates except rats of the genus *Rattus*, mice of the genus *Mus* and birds) must not be held outside the LARC for more than **12 hours** unless specified in the IACUC-approved protocol.

C. No other vertebrate animals are to be held outside of the LARC for more than **24 hours** unless specified in the IACUC-approved protocol.

D. Any site where animals are held for times exceeding those specified under Sections 2.B. and 2.C. is, by law, considered an animal housing facility and must comply with the regulations outlined within the most recent versions of "The Guide for the Care and Use of Laboratory Animals" (The Guide) of the National Research Council of the National Academies, Washington, D.C., the U.S. Department of Agriculture (USDA),[1] and the National Institutes of Health (NIH) Office of Laboratory Animal Welfare (OLAW).[2] When animals are to be housed in a laboratory, the PI will be responsible for following the regulations governing housing facilities, maintaining the laboratory in a manner that complies with those regulations, and maintaining appropriate records as defined by the regulations.

E. The LARC staff will make every effort to house animals according to the PI's specifications. However, the LARC must comply with TTUHSC policies, rules from "The Guide", and federal laws and regulations governing the sizes of cages and numbers of animals per cage, which shall supersede a PI's specifications.

F. All animal rooms must be maintained by LARC personnel unless otherwise authorized by the Institutional Veterinarian or designee. If a PI's study involves the use of special diets or has other requirements, PI's technicians may implement those husbandry requirements with the approval of the Institutional Veterinarian or his/her designee.

3. Accountability

Animals must be recorded as "used on protocol" when issued to the requesting PI.

A. Rodents: The language of the approved protocol will determine how rodent mothers, pups, litters, etc. are counted against the protocol census.

1. If a PI plans to perform experiments on unweaned pups, one mother with pups will be counted as one litter. All weaned pups from those litters will be counted against the protocol census.
2. In a breeding colony, unweaned pups are not counted against the protocol census. Once pups are weaned, they must either become part of the breeding colony, be transferred to a research or training protocol, or be euthanized.

B. Non-Rodents: Zebrafish offspring from breeding colonies that are used in studies must be included in the protocol census. All other offspring are to either become part of the breeding colony or euthanized.

References

1. USDA Animal Welfare Act, Animal Welfare Regulations, Animal Welfare Policies; Animal and Plant Health Inspection Service, U.S. Department of Agriculture. www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalwelfare
2. OLAW regulations on housing facilities: http://grants.nih.gov/grants/olaw/olaw.htm

Policy 4: Veterinary Care

1. Institutional (Attending) Veterinarian

The Institutional Attending Veterinarian (IVet), located at the TTUHSC Lubbock campus, is responsible for the health and welfare of all animals used in teaching, testing, and research at TTUHSC facilities. The IVet is responsible for managing the program of Veterinary Care. The IVet reports to the TTUHSC Institutional Official and is a voting member of the IACUC

2. Qualifications of the IVet

The IVet is the TTUHSC Attending Veterinarian and must meet the minimum requirements as defined by the Animal Welfare Act and other Federal laws, Guidelines, or Policies. These requirements include:

- Being a graduate of an AVMA-accredited Veterinary School or foreign equivalent
- Having appropriate training and/or experience in Laboratory Animal Science and Medicine and the care and management of the species being utilized; Maintaining a valid license to practice veterinary medicine in the US
- Maintain USDA Veterinary Accreditation in the State of Texas

3. Duties of the IVet

The IVet oversees the Program of Veterinary Care, which includes the oversight and management of the following:

- Animal procurement, transportation, and housing
- Preventive medicine (including quarantine, biosecurity, and animal health surveillance)
- Clinical disease, disability, or related health issues
- Protocol-associated disease, disability, or other sequelae
- Animal surgery and post-operative care
- Pain and distress
- Anesthesia and analgesia
- Euthanasia

A. has overall responsibility for animal health and well-being at TTUHSC. The IVet may assign or direct a Clinical Veterinarian, a veterinary technician or designee duties as need to ensure adequate oversight of animal health.

B. may suspend animal use and, if necessary, quarantine, treat, or euthanize animals, when deemed in the best interest of the animal(s). If suspension or quarantine occurs, the IVet shall notify the Principal Investigator (PI) immediately. The IVet will also notify the PI and IACUC Chair, in writing, within 3 business days.

C. will oversee the Laboratory Animal Resource Center (LARC) staff and oversee its day-to-day functions.

D. will provide veterinary oversight and may assist on all approved research and training projects and may assign appropriate LARC personnel to assist in procedures for any current protocol.

1) The IACUC will provide general oversight to ensure that all personnel are properly trained, the IVet will provide specific training and oversight as needed or upon request by researchers.

2) Records of staff qualifications, training, and experience with each species will be kept by the Research Integrity Office. These records will be made available for annual IACUC review and assurance that personnel are adequately trained.

3) The IVet and LARC personnel supervised by the IVet have TTUHSC IACUC approval to work on all IACUC-approved protocols without being individually named on the protocol. This allows the IVet and LARC staff to freely assist any investigator with animal procedures in the approved protocol. However, this does not allow the IVet and LARC staff to change any animal procedure without IACUC approval.

E. will provide "on-call" animal health support on an ongoing basis including weekends, holidays and after regular business hours, or will arrange back-up veterinary care in the absence of the IVet.

F. will participate as a voting member of the TTUHSC IACUC.

G. will review all protocols and those amendments that affect personnel or animal welfare that are submitted to the IACUC. The IVet will work with the IACUC member assigned as a reviewer to require revisions as necessary.

H. will supervise Clinical Veterinarians at all TTUHSC animal resource centers and their satellites on any TTUHSC campus.

4. Clinical Veterinarians

TTUHSC retains a local veterinarian at each of its campuses that possesses an animal facility. Each Clinical Veterinarian shall have authority to carry out the duties described below.

The Clinical Veterinarian at each campus is directly responsible to the IVet and acts in conjunction with the IVet and the IACUC to ensure the health and welfare of animals maintained there.

5. Qualifications of Clinical Veterinarians

All Clinical veterinarians are required to meet current training and licensure requirements, to be determined by the Institutional Veterinarian.

6. Duties of Clinical Veterinarians

In the event of a conflict between any action taken by a Clinical Veterinarian and the IVet pursuant to carrying out the duties below, the IVet exercises final decision-making authority.

If a Clinical Veterinarian anticipates being unavailable, the Clinical Veterinarian shall contact the IVet to make arrangements for coverage at that particular campus.

A. **Lubbock** - duties to be carried out in the absence or unavailability of the IVet.

The Clinical Veterinarian shall:

1) Respond promptly to emergency health matters at the request of IVet, LARC technician(s)/staff, or research staff at the TTUHSC campus in Lubbock.

2) Provide "on-call" animal health support on an ongoing basis including weekends, holidays, and after regular business hours for the LARC.

3) Suspend animal use, and, if necessary, quarantine animals at the LARC, when deemed in the best interest of the animal(s). If suspension or quarantine occurs, the Clinical Veterinarian shall immediately notify the IVet, or when unavailable, the LARC, who in turn shall immediately notify the PI. The IVet and/or the LARC will also notify the PI and IACUC Chair, in writing, within 3 business days.

B. **Other TTUHSC Campuses** (including campuses at Amarillo and Abilene).

The Clinical Veterinarian shall:

1) Perform an inspection of the applicable TTUHSC animal resource center at least once per week to observe animals for general health and to note any issues that involve the facility, including sanitation, but also to include any other issues that should be addressed by the IVet. The Clinical Veterinarian shall provide (in writing) any findings of non-compliance to the IVet. Significant infractions will be relayed immediately to the IVet.

2) Meet with the IVet or designee on an as-needed basis to discuss issues related to the applicable TTUHSC animal resource center and for training.

3) Respond promptly to emergency health matters at the request of the IVet, technician(s)/ staff at that TTUHSC campus animal resource center.

4) Suspend animal use, and, if necessary, quarantine animals at the LARC, when deemed in the best interest of the animal(s). If suspension or quarantine occurs, the Clinical Veterinarian shall immediately notify the IVet, or when unavailable the LARC, who in turn shall immediately notify the PI. The IVet and/or the, LARC, will also notify the PI and IACUC Chair, in writing, within 3 business days.

5) Provide veterinary oversight on approved research and training projects as needed at the applicable TTUHSC animal resource center.

6) Provide "on-call" animal health support on an ongoing basis including weekends, holidays and after regular business hours at that TTUHSC campus animal resource center.

Policy 5: Pain Categories for Experimental Protocols

Each protocol submitted to the IACUC is assigned one of the following pain categories, which are based on the column headings from APHIS Form 7023a *United States Department of Agriculture (USDA) Annual Report of Research Facility*. There is no *Category A.*

Category B

Definition - Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes. This category is automatically assigned to breeding and holding protocols.

Category C

Definition - Teaching, research, experiments, or tests involving no pain, distress, or requirement for pain-relieving drugs.
Examples:
• Non-dangerous procedures such as injections of small amounts of nonionic substances or blood sampling.
• Observation of natural behavior.
• Behavioral testing without significant restraint or noxious stimuli.
• Standard methods of euthanasia that induce rapid unconsciousness, such as an anesthetic overdose.

Category D

Definition - Experiments, teaching, research, surgery, or tests that result in pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs are used.
Examples:
• Experiments on completely anesthetized animals that do not regain consciousness.
• Survival surgery with appropriate anesthetic, analgesic, or tranquilizing drugs.

Category E

Definition - Experiments, teaching, research, surgery, or tests that result in pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs cannot be used, because such use would adversely affect the procedures, results, or interpretation of the outcome.

Protocols in Category E may receive scrutiny from external sources; therefore, the protocol must include (A) an explanation of the procedures producing pain or distress in these animals and (B) the reasons palliative drugs cannot be used.

Policy 6: Protocol Review and Approval Procedures

1. Background

Any use of live vertebrate animals (including teaching, research, testing, experimentation and exhibition) that occurs under the auspices of TTUHSC (regardless of funding source) must first be reviewed and approved by the IACUC.

The TTUHSC IACUC meets on the second Friday of each month. Items for IACUC consideration must be received by the submission deadline available on the IACUC website to be placed on the next month's meeting agenda.

2. General Requirements for Animal Use at TTUHSC

Before utilizing live animals at TTUHSC,

A. The Principal Investigator (PI) I must have a faculty appointment at TTUHSC (as defined by TTUHSC Operating Policy 73.08), or Texas Tech University (as described in IACUC Policy #2 and the *Memo of Understanding between TTU and TTUHSC*) unless otherwise approved by the TTUHSC Institutional Official.

B. The PI must have either:

1) an animal protocol approved by the TTUHSC IACUC[10]; or,

2) written approval of the Institutional Veterinarian (IVet) to transfer animals into the Laboratory Animal Resources Center (LARC) Holding Protocol. In this case, the PI must have a protocol already approved by another agency/institution's IACUC, and provide the approved protocol to the TTUHSC IACUC. Animals on the LARC Holding Protocol may not be utilized until the TTUHSC IACUC approves the protocol.

C. All ordering or import of animals must be processed through the LARC and is subject to IVet approval.

3. Submission of the Initial Review Submission Form (Start of Study)

A. The PI must submit a completed Initial Review Submission Form in iRIS for IACUC consideration and review. This e-form includes the attached IACUC Application describing the protocol information.

B. After submission of an Initial Review Submission Form, the following items must be completed before animal use may begin:

1) The protocol must be approved by the IACUC.

2) IACUC-required online CITI training must be completed by all personnel listed on the protocol.[6] Contact the IACUC staff for details.

3) All personnel listed on the protocol must be enrolled in the Occupational Health and Safety Program (OHSP).[5] The PI is responsible for ensuring that this requirement is met.

4) A protocol for research involving hazardous chemicals and biological materials and recombinant or synthetic nucleic acid molecules must be approved by the TTUHSC Institutional Biosafety Committee (IBC) or TTU IBC (for School of Veterinary Medicine IACUC protocols).

5) In order to access TTUHSC Vivaria, all personnel on the protocol must complete a LARC Orientation with the facility supervisor.

4. General Requirements for Animal Use

A. Students or temporary personnel working in laboratories must participate in the training program before working with animals and must be listed on the PI's protocol(s).

B. The PI must ensure individuals listed on protocol(s) receive the appropriate IACUC training[10], are qualified and competent in the animal procedures or are closely supervised by qualified and experienced personnel[8], and are approved to handle animals by the IACUC (except as noted in section 4.D. below).

C. Access to TTUHSC Vivaria will be given only to persons:
   1) listed on an IACUC-approved protocol;
   2) with completed IACUC-required training;
   3) enrolled in the Occupational Health and Safety Program; and,
   4) after completion of the LARC Orientation by the facility supervisor.

D. Persons not listed on an IACUC-approved protocol may not handle the animals. The only exception is those persons who are participating in training under an IACUC-approved, designated Training Protocol (i.e., not a PI's research or breeding protocol).

5. Continuing Review of Approved Protocols

The IACUC's review responsibilities do not end with initial approval of the protocol. The IACUC is obligated by both AWAR and the PHS Policy to conduct ongoing reviews of protocols[7] which serve to ratify the decisions of the IACUC on the current status of the protocol.

A. Annual Status Reports (ASR)

The PI will submit an ASR e-form in iRIS for review[4] and approval by the IACUC every year before the anniversary month of the latest approval date of the protocol. The ASR may be without changes requested to the IACUC Application, or the ASR may be with changes requested to the IACUC Application.

An ASR without changes does not require a revision of the approved IACUC Application. An ASR with changes must be accompanied by an Amendment submission describing the changes being requested. The Amendment must include an attached and revised version of an IACUC Application. The PI must submit all required forms with sufficient time to be placed on the IACUC agenda for consideration of approval before the study anniversary date.

B. Three-Year Renewals

Every third year, the PI must submit the IACUC 3-Year Renewal e-form in iRIS and update the last approved version of the IACUC Application with a revised IACUC Application attached for review and approval by the IACUC before the anniversary month. This will count as the ASR for that year. Renewals must be submitted with an update of the last approved version of the IACUC Application. The PI will receive an iRIS e-notification before the anniversary month, and must update the last approved version of the IACUC Application.

If the PI fails to submit all required forms with sufficient time to be placed on the IACUC agenda for consideration of approval before the study anniversary date, then the study will expire, and the protocol must be resubmitted as an Initial Review Submission Form for reinstatement.

C. Non-Use Administrative Termination of Protocol

The 114th Congress (2015-2016) H.R. 34 - 21st Century Cures Act, Section 2034 (d), mandates federal efforts to reduce administrative burden for researchers while maintaining the integrity and credibility of research findings and protection of research animals. Therefore, if animals have not been ordered or used during the previous 36 months since the Initial Review Submission or Three-Year Renewal was approved, the study will be administratively terminated. If administrative termination of a "non-use" protocol occurs, PIs may submit an *Initial Review*

Submission Form, and the IACUC will consider the judicious use of animals in research and will assess the scientific importance of the study.

6. Post-Committee Review Process

After submitted protocols and amendments are presented and discussed at an IACUC-convened meeting, the committee members present will vote to either a) approve, b) require modifications to secure approval, or c) withhold approval.[1]

A. When the IACUC requires modifications of a protocol in order to secure approval, the members will vote to follow one of the procedures described below:

1) A second Full Committee Review (FCR), following the procedures delineated above.

2) A Designated Member Review (DMR), if approved unanimously by all members at the meeting, following the procedures described in Policy #7. However, if any member calls for FCR of the modifications, such modifications can only be reviewed and approved by FCR.

3) Minor modifications may be confirmed by IACUC administrative staff, if approved by the designated members (if DMR) or unanimously by all members at the meeting when the protocol was presented (if FCR).

B. Procedures related to animal care and use, housing and management should be continuously evaluated, and when indicated, should be refined or replaced.[2] During the continual review of protocol procedures, investigators may be asked to make changes in their protocol due to regulatory changes and advances in veterinary standards of care.[8]

C. Research described in an NIH grant application[10] must be congruent with any corresponding TTUHSC IACUC-approved protocol as determined by the Institution. A one-to-one relationship between the grant and the approved protocol is not required, and more than one protocol may be associated with one grant and vice versa.[9,11]

7. Amendments

A. Once a protocol has been approved, any and all changes requested must be submitted as an IACUC Amendment in iRIS. The Amendment must include an attached revised IACUC Application. All proposed changes must be approved by the IACUC or designee in writing before implementation by the PI.[10].

B. Certain additions, deletions, and/or changes to an approved protocol may occur via the Administrative Approval process as outlined in Policy #8.

C. Significant changes to an approved protocol may occur via the Veterinary Verification and Consultation[3] process as outlined in Policy #8.

References

1. *Animal Welfare Act and Animal Welfare Regulations* § 2.31
2. *AVMA Animal Welfare Principles*
3. *Guidance on Significant Changes to Animal Activities* NOT-OD-14-126
4. *Guide for the Care and Use of Laboratory Animals*
5. *Institutional Animal Care and Use Committee Guidebook*
6. *Standards and certification process for humane handling, care, treatment, and transportation of animals.* 7 U.S.C. § 2143
7. Silverman, J., Suckow, M.A. & Murthy, S. (2014). *The IACUC Handbook*, 3rd ed.
8. *US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training*
9. *Verification of IACUC Approval.* NIH Grants Policy Statement 4.1.1.2.
10. *What Investigators Need to Know About the Use of Animals*. NIH Publication No. 16-6009
11. *NOT-OD-22-005 Notice of Clarification of Institutional Responsibilities - NIH Grant to Protocol Congruence Review*

Policy 7: Designated Reviews

1. Background

Only two protocol review methods fulfill USDA and PHS requirements - full committee review (FCR) and designated member review (DMR). Ordinarily, for FCR the IACUC members (during a convened meeting) review and vote on the acceptability of animal use protocols submitted by a principal investigator (PI). For DMR, at least one member of the IACUC shall review those protocols and have the authority to approve, require modifications (to secure approval) or request FCR. DMR may be used to secure approval for (1) new or renewing full protocols and amendments that require immediate evaluation, (2) Annual Status Reports (ASR), or (3) protocols that have first undergone FCR.

2. Designated Member Review

This section will describe the DMR process as applied to submissions of either full protocols or amendments that require immediate evaluation. The use of this process must be justified.

A. The PI shall submit an appropriately completed IACUC protocol form and a separate email request for a DMR to the IACUC staff**.** The email request must contain a justification for conducting a DMR.

B. The IACUC staff will notify the IACUC Chair (or designee) of the request. The Chair will determine whether or not to grant the request for a DMR. The IACUC Chair (or designee) will advise the PI at this step only if the DMR request will not be forwarded to IACUC members.

C. If the DMR request is granted, the Chair (or designee) will notify the IACUC staff to poll the IACUC members. Each IACUC member will have access to the item submitted for IACUC consideration, the request for DMR, and any other necessary information concerning the proposed research project for their consideration about a DMR. Each IACUC member will have an opportunity to call for FCR of the protocol rather than the DMR.

D. IACUC members must reply to the IACUC staff within three business days of initiation of the poll. If an IACUC member has not responded within that time, the lack of a response will indicate their approval of the request for DMR. Records of the polling will be maintained within the IACUC office. The IACUC staff will notify the investigator of the IACUC's decision only if a DMR will **not** occur.

E. If the results of the poll support the DMR, the Chair (or designee) will initiate the DMR process by appointing a subcommittee consisting of not less than one designated reviewer and the Institutional Veterinarian or another veterinarian. The subcommittee is authorized to review and approve, require modifications, or request FCR of the submitted item.

F. Once the DMR process is complete, the designated reviewer will notify the IACUC staff of the review decision and any recommendations. The IACUC staff will notify the IACUC Chair and investigator of the designated member's decision and of any recommendations made.

G. The IACUC will be notified of the results of the DMR at the next convened IACUC meeting.

3. Annual Status Report

This section will describe the DMR process as applied to the review of Annual Status Reports.

The Annual Status Report (ASR) is a crucial element of the Institutional Animal Care and Use Committee's (IACUC) assessment of the handling of animals. The ASR provides the Committee with a status report on animal usage within the previous twelve-month period, and a plan for animal research during the next twelve-month period. In addition, in order to remain compliant with USDA rules, those protocols using USDA-regulated species must have the ASR submitted and approved by the one-year anniversary date of the last approval.

The TTUHSC IACUC goes one step further and requires all protocols to submit an ASR whether they have USDA-regulated species or not, as a method of Post Approval Monitoring (PAM). As calendar dates for IACUC meetings are based on the second Friday of a given month, it is theoretically possible that the deadline for receipt of an ASR might pass prior to a meeting of the full committee. Therefore, the deadline for submission might be a full month prior to the usual deadline to avoid a lapse of approval.

In order to facilitate this process, the IACUC will routinely review ASRs by DMR. All ASRs will be posted to an IACUC agenda. Additionally, all study documents will be available (in iRIS) for every IACUC member for review. Any IACUC member may call for FCR prior to finalization of the agenda. At least one member of the IACUC will be assigned by the Chair (or designee) to review the ASR and have the authority to approve, require modification to secure approval or request FCR prior to the previous approval's anniversary date. If there are multiple designated reviewers, there must be unanimous approval. If agreement is not unanimous, the protocol will automatically go to FCR. All deliberations and results will be documented in the IACUC minutes.

4. DMR following Full-Committee Reviews

This section will describe the DMR process as applied to protocols that have first undergone FCR (Post-committee review process).

Following the presentation and associated discussion of a protocol, amendment or ASR at FCR, the IACUC members who are present will vote to either a) approve, b) require modifications to secure approval, or c) withhold approval. Modifications of these items may be designated as either minor (modifications that do not involve animal health or welfare) or major (modifications that do involve animal health or welfare). While minor modifications will be confirmed by DMR and processed administratively (see Policy #7), approval of required major modifications will be delayed until appropriate changes are approved via DMR.

Specifically, the DMR will occur subsequent to FCR, if approved unanimously by members at the meeting in which the matter is discussed. If any member calls for a second FCR to consider the modifications further, then the DMR process will not be followed. Such modifications will only be reviewed and approved by FCR.

Policy 8: Administrative Approvals of Protocol Amendments

1. Background

The Assurance between TTUHSC and Public Health Services for the IACUC allows administrative approval for protocol amendments that are solely to:

A. Add, delete, or change a title of a study;
B. Add, delete or change personnel other than the Principal Investigator;
C. Add and/or delete location(s);
D. Add and/or delete animals of species (rats and mice only) approved to be used on the protocol when the increase is no greater than 10% of the approved number of animals;
E. Add, delete or change a funding source.

The Principal Investigator (PI) is responsible for the submission of the completed and signed amendment form to the IACUC staff. Final approval of an amendment for addition of personnel will not be given until all personnel have received appropriate training and have enrolled in the Employee Health program (EHP).

2. Addition of Personnel (other than PI)

Once the IACUC staff receives an amendment that includes addition of new personnel, the person(s) listed in the amendment and the PI will be notified of the following items:

A. Personnel **shall not work** with animals until they have completed the required on-line training course(s) at www.citiprogram.org and this has been verified by the IACUC Administrator and/or his/her designee. Required training includes completing the Investigators, Staff and Students course and the appropriate species-specific modules (dependent upon each individual protocol).

B. Personnel also **shall not work** with animals until they have enrolled in OHS/EHP. The IACUC Administration and/or his/her designee must receive verification of this enrollment from the EHP nurse on the campus the personnel will be working.

3. Addition of Location

Addition of new location(s) for the use of animals may be administratively approved after the PI has submitted an amendment indicating a request for location change. This location **shall be verified in writing** by the Institutional Veterinarian or designee as allowable before the site may be used to conduct animal work. All non-LARC locations are subject to IACUC approval and must be inspected by the IACUC before animal work may commence. The Institutional Veterinarian (or designee) shall have 24/7 access to all locations where animal work is carried out.

4. Addition of Animal Numbers

An increase of up to 10% in the number of rats and/or mice (but not other species) approved in the **original** protocol may be approved administratively after the PI has submitted an amendment indicating a request for additional animals. This number **must be verified** by IACUC as allowable before additional animals may be put onto the protocol by purchase or transfer.

5. Addition of Funding Source

Addition of a new or different funding source may be administratively approved. However, if the funding source requires a grant/protocol comparison, then the change may be administratively approved only after the assigned reviewer **provides written verification** that the comparison is satisfactory.

6. **VVC – Veterinary Verification and Consultation**

Certain specific significant changes (outlined in OLAW Guidance #NOT-OD-14-126 and further specified in this policy) may be approved by the Institutional Veterinarian (IVet), with proper consultation and review of the approved protocol.

The IVet is not conducting DMR, but is serving as a subject matter expert to verify that compliance with the IACUC-reviewed and IACUC–approved policy is appropriate for the animals in various circumstances. Consultation with the IVet will be documented. The IVet may refer any request to the IACUC for full-committee review. Documentation of the Consultation will be forwarded to the IACUC Administrator for attachment to the protocol. The PI shall submit a protocol amendment within one month of the Consultation. This amendment may be approved as needed by Designated Member Review by an eligible IACUC member who is not the IVet. The following changes may be handled administratively through the IVet and IACUC staff:

A. Anesthesia, analgesia, sedation, or experimental substances;
B. Euthanasia to any method approved in the AVMA Guidelines for the Euthanasia of Animals; and,
C. Duration, frequency, type, or number of procedures performed on an animal.

VVC cannot be used to add new procedures or study objectives to a previously approved protocol. In addition, modifications to existing procedures with the possibility that animal welfare will be compromised must undergo committee review. In particular, the following changes require committee review:

A. Change from non-survival to survival surgery;
B. Changes resulting in greater pain, distress, or degree of invasiveness; and,
C. Changes that affect personnel safety.

Reporting to IACUC

All administratively approved amendments will be placed on the agenda for the next scheduled meeting of the IACUC for informational purposes. All administrative approvals take effect when verification of all requirements is completed and written notification from the IACUC or their designee is sent to the PI.

Policy 9: Review of Grant Content with IACUC Protocol

1. Purpose

The NIH and NSF require verification of research protocols by grantee institutions to ensure compliance with the terms of the award. Further, the Institution is responsible for ensuring that the information that the IACUC reviews and approves is congruent with what is in the application or proposal. This may require comparison of the proposed or funded research protocol with approved protocols of Institutional Review Boards. Currently, the NIH and NSF require confirmation that proposed experiments involving animals have been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). This policy establishes a method of assuring that the TTUHSC IACUC complies with these requirements.

2. Definitions and Mandates

A. **Congruence**, as opposed to equivalence or approximation, is a relation that implies a kind of equivalence, though not complete equivalence.

B. **Change in scope**. Although not defined specifically by OLAW, potential indicators of a change in scope include:

1) Changes in specific aims of the award.

2) Changes in animal model.

3) Changes from the approved use of live vertebrate animals.

4) Shifts of the research emphasis from one disease area to another.

C. **Content that should be compared**:

1) Species

2) Animal numbers proposed

3) Procedures, including minimization of pain and distress.

3. Protocol Procedures

The IACUC is a standing institutional committee specifically charged with the protection of animals used in research. Because it has no direct knowledge of grants filed or funded, this policy will be implemented with the cooperation of the Office of Sponsored Programs, as follows.

A. The PI will ensure that each NIH/NSF grant submission will be supported by a single corresponding IACUC protocol (1:1 grant to protocol ratio).

B. The Office of Sponsored Programs will:

1) Notify the IACUC of a newly approved or renewed NIH or NSF grant when TTUHSC is the primary recipient and animals will be used.

2) Supply a file containing the relevant portions of the grant to the IACUC staff, if the PI is unavailable.

C. The IACUC Chair or the IACUC staff will assign the grant review to a member of the IACUC (generally the same member of the committee assigned to review the IACUC protocol).

D. The IACUC member will complete a timely review of the grant document(s) and the protocol and determine the general level of congruence.

1) If no change in scope is noted, this result will be documented in iRIS.

For instances where there may be a change in scope:

2) In cases where the grant describes animal experiments that are not part of an approved protocol, but no animals have been used:

a) The Reviewer will ask the PI for clarification or request that the protocol be amended to be consistent with the grant.

b) The Principal Investigator will be responsible for notifying the funding agency and providing documentation of such to the IACUC if any procedures will not be conducted as originally proposed.

3) In cases where the grant describes animal experiments that are not part of an approved protocol (or diverge significantly from animal experiments that have been approved) and animals have been used:

a) The matter will be handled in accordance with Policy 10 ("Complaints of Mistreatment of Animals and Policy Noncompliance at TTUHSC").

b) The IACUC Chair and Institutional Veterinarian will investigate and take necessary action, which may include suspension of animal use (Policy 10, Sections 6-8).

i. If no violation or a minor violation is found, the matter will be addressed in accordance with Policy 10, Sections 6 and 7.

ii. If a major violation is found, the matter will be referred to the IACUC Protocol Violations Subcommittee for appropriate action. The Subcommittee will report to the IACUC, which will determine final action (Policy 10, Section 8.B.).

4) In cases where a procedure is described in an approved protocol but is not described in the grant, whether or not animals have been used, the reviewer will first determine if the procedures in the protocol significantly extend or modify the scope of the grant. If necessary, the reviewer will then ask the PI for clarification or request that the protocol be amended in order to be consistent with the grant. If amended, the changes will be reported to the NIH.

Policy 10: Reporting of Adverse Events, Animal Welfare Concerns, Policy Non-Compliance, and Unexpected Outcomes

1. Definitions

- **Adverse event** – An unexpected incident that negatively affects the health or welfare of animals.
- **Animal welfare concern** – A condition or situation that has the potential to jeopardize the health or well-being of animals, including suspected mistreatment and misuse.
- **Mistreatment/Misuse -** Any intentional action which results in wrongful or abusive treatment of an animal.
- **Noncompliance -** Violation of TTUHSC policy or noncompliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy), the USDA Animal Welfare Act Regulations (USDA AWAR), and other applicable federal, state, and local laws or regulations governing animal care and use. Any deviation from policies or approved procedures without prior IACUC or IVet approval.
- **Unexpected outcome –** An unanticipated result of IACUC-approved animal activities that results in negative effects to animal health or welfare. Examples of reportable unexpected outcomes may include and are not limited to:
    - Animal morbidity or mortality occurring at a higher frequency than expected.
    - Unanticipated debilitating defects discovered after creating or breeding genetically modified animals.
    - A non-research event that is unanticipated or due to a facility, physical plant, equipment, or personnel failure, malfunction, or mistake.

2. Who MUST report

Any Principal Investigator or Co-Investigator upon learning of any reportable condition pertaining to their protocols as outlined in Section 4 below

3. Who MAY report

Any person, including any University employee, student, volunteer, or member of the general public, may report concerns involving the care and use of animals at TTUHSC. Such concerns may be reported anonymously. If the complainant identifies him/herself, protection of his/her identity will be maintained to the extent possible within the legitimate needs of law and the investigation.

4. When and what to report

Internal Reporting. Adverse events, Animal welfare concerns, Mistreatment/Misuse (suspected or observed), instances of Noncompliance, and Unexpected outcomes **must** be promptly reported to the IACUC. Some events may require immediate action by the animal program or veterinary staff to protect animal health and welfare in addition to IACUC reporting. Based on federal requirements, the IACUC has identified several kinds of reportable incidents that may serve as examples to guide individuals in making their determinations. These incidents include, but are not limited to, the following:

- a condition that is or may be a threat to the health or safety of animals, the conduct of animal-related activities or protocol modifications without prior IACUC review and approval
- continuing activities past the protocol expiration date
- the conduct of activities by uncertified and/or unlisted personnel
- continuing noncompliance
- inadequate veterinary care
- accidents or errors affecting animals or workers
- equipment failures
- natural disaster
- inappropriate euthanasia techniques and/or failure to confirm euthanasia
- a significant human health issue directly-related to the animal care and use program.

For clarity, examples of unexpected outcomes that do *not* require reporting include:

- Death or morbidity of animals described as expected in the approved IACUC protocol.
- Mortality resulting from surgical complications anticipated in the approved protocol at or below the rate anticipated in the approved protocol.
- Injury/illness unrelated to approved procedures and being treated by a veterinarian.
- Since the chance of mortality increases as a function of age in all animals, the death of aged animals due to natural causes or required euthanasia due to age-related conditions (i.e., severe arthritis)

5. How to report

A. Reports are encouraged to be made in writing to assure a clear understanding of the issues raised, but may be made verbally. Such reports should contain as much specific information as possible to allow for a full investigation and proper assessment of the nature, extent, and urgency of the concern. Information should include, but need not be limited to, the nature, date, time, and location of the occurrence; the person(s) against whom the allegation is being made; and any supporting documentation.

B. A complaint may be reported either orally or in writing, to any member of the IACUC. Complaints may also be sent by email to larc@ttuhsc.edu or anonymously by visiting www.ethicspoint.com or calling 866.294.9352.

C. If the complaint is against the IACUC Chair, then it should be reported directly to the Institutional Official (IO). All other complaints should be reported to the IACUC Chair.

6. Reporting and processing of complaints

A. If the Institutional Veterinarian (IVet) is the subject of the complaint, the IO will appoint an individual to conduct the initial investigation.

B. If the complaint includes issues regarding animal welfare, then the IACUC Chair will inform the IVet and both will be involved in further investigation of the complaint.

C. The IACUC Chair will inform in writing the person who is the subject of the complaint (and the PI, if the PI isn't the direct subject of the complaint) that a complaint has been received and provide a summary of the complaint.

D. The IACUC Chair will also notify the Complainant (in writing) that the complaint has been received, unless the Complainant chooses to remain anonymous or does not provide sufficient contact information.

E. All records of the complaint (including all results and communications) shall be documented and maintained in IACUC files for a minimum of three years.

7. Evaluation of complaints and immediate action steps

Upon receipt of a reported concern, the IACUC Chair, in consultation with the IVet, will evaluate the complaint to determine whether there is sufficient information to investigate further. As much information as is reasonably needed will be collected, which may entail review of documents, inspection of the facilities, and/or discussions with pertinent individuals.

A. If no violations are found, then no further action after investigation of the concern will take place, other than to inform (in writing) both the Complainant and the subject of the complaint of the finding and the recommendation that no further action will be required. If the Complainant disagrees with the response from the IACUC Chair, then the complaint will be further processed as a "Disputed Violation" and handled as described below in Section 8.B.

B. If mistreatment of animals is found, the Chair and IVet shall take immediate steps to ameliorate the problem and protect the animals. Such ameliorative steps may include veterinary medical intervention, confiscation of the

animals, and/or suspension of activities. In some cases, involvement by the IO, legal counsel, and other University officials (e.g., Department Chair) may be required at the outset of the investigation.

C. If suspension of the use of animals is implemented, the IVet will notify the person who is the subject of the complaint (and the PI, if the PI is not the direct subject of the complaint) in writing and in a timely manner.

8. Further actions to be taken after a violation has been found

A. Minor Violation(s)

If, following the initial investigation, it is determined that a minor violation (consisting of one instance of policy noncompliance but no inhumane treatment of animals) has occurred, then all the following will occur.

1) The IACUC Chair will inform in writing the person who is the subject of the complaint and his/her Department Chair or supervisor of the findings and recommendations and of any required remediation, including a time frame in which corrections are to be completed.

2) The IACUC Chair is responsible to report the complaint, the findings and recommendations, and any remedial action to the IACUC.

3) The IACUC Chair and/or IVet will be responsible to verify that the remediations have been completed in a timely manner.

4) If either the Complainant or the person who is the subject of the complaint disagree with findings, or if the person who is the subject of the complaint disputes or refuses the remedial actions, the complaint will be further processed as a "Disputed Violation" as described below in Subsection B, below.

B. Major Violation(s) and/or Disputed Violations

If, after the initial investigation, it is determined that a major violation has occurred (consisting of either two or more instances of noncompliance or the inhumane treatment of animals) or the initial findings are disputed (as discussed in sections 7.A and 8.A.4), then all the following will occur.

1) The IACUC Chair will schedule a meeting of the IACUC Protocol Violations Subcommittee (Subcommittee) within 10 business days from receipt of IVet's findings and recommendations. The Subcommittee is a standing committee with members appointed by the IACUC Chair. All Subcommittee members will be expected to attend this meeting. If essential duties require absences, a majority of the standing subcommittee members must be present.

2) The Subcommittee will review the complaint and the initial findings and recommendations of the IACUC Chair and the IVet and obtain additional information as needed.

a) The Complainant may be requested to meet with the Subcommittee. If the Complainant is unwilling or unable to make a personal appearance, the Complainant may submit a written statement to the IACUC Chair prior to the meeting. The IACUC Chair shall provide a copy of the statement to the person who is the subject of the complaint.
b) The person who is the subject of the complaint will be given an opportunity to meet with the Subcommittee to respond to the complaint and ask questions of the Complainant and any other witnesses. At least three (3) business days prior to the meeting, the person who is the subject of the complaint will be provided a copy of any written complaint or other written documentation pertaining to the complaint. If the person who is the subject of the complaint is not the Principal Investigator (PI), the complaint may be forwarded, at the discretion of the IACUC Chair or Vice Chair, to the PI or other supervisor directly responsible for the animal(s) subject to the complaint. If a verbal complaint is made, the person who is the subject of the complaint will be informed of the substance of the complaint at least three (3) business days prior to the meeting.

c) The Complainant and the person who is the subject of the complaint may have an advisor at the meeting with Subcommittee, provided that written notice is given to the IACUC Chair at least two (2) business days in advance of the meeting with the Subcommittee. Advisors are present in an advisory capacity only and are not permitted to speak or present information directly to the Subcommittee.
d) If the Complainant and/or the person who is the subject of the complaint elects not to meet with the Subcommittee, the complaint will be reviewed based on information available, and a recommendation will be made by the Subcommittee. No inference may be drawn against the Complainant and/or the person who is the subject of the complaint for failure to appear before the Subcommittee.
e) At the meeting, the Subcommittee may call the IVet or any other witnesses as it deems necessary.
f) When the Subcommittee concludes that all pertinent information has been received, anyone who is not a voting member of the Subcommittee shall be excused, and the Subcommittee shall discuss, deliberate, and prepare its findings and recommendations. By majority vote of those present, the Subcommittee will determine whether mistreatment of animals or policy noncompliance has occurred (findings) and make recommendations. If the findings and recommendations are not unanimous, opinion(s) may be written and attached by those who differ with the majority's findings and recommendations.

3) The Subcommittee will present its findings and recommendations, including any differing opinion(s), to the IACUC at the next scheduled meeting. The IACUC will then determine final action(s) to be taken. IACUC action(s) may include, but is not limited to:
   a) Finding of no violation and/or the complaint was not substantiated, and filing this written notification in the PI's IACUC protocol file. If the person who is the subject of the complaint is not a PI, the written notification will be filed in the IACUC file;
   b) Reprimand to the person who is the subject of the complaint, which may include notice to the PI that if the same or similar circumstances which were the basis of the complaint are found to continue, this could result in suspension or complete revocation of approval of all protocols relevant to the complaint under which animal(s) were obtained;
   c) Suspension of the protocol for a stated period of time, or immediate revocation of the protocol approval; or,
   d) Recommendation to the IACUC IO to suspend all LARC access of the person who is the subject of the complaint for a stated period of time, or to take other action as the IO deems appropriate.

4) The IACUC Chair will report in writing, as appropriate, to the following individuals:
   a) The Complainant (if contact information is known);
   b) The person who is the subject of the complaint;
   c) The Departmental Chair or supervisor of the person who is the subject of the complaint; and,
   d) The IO.

5) The IO will report the findings and actions taken to all appropriate external organizations and/or governmental agencies.

Policy 11: Breeding Colonies

1. Purpose

The purpose of a breeding colony protocol is to generate animals for use in approved experimental protocols. Breeding colony protocols must be submitted *separately* from experimental protocols that use animals from the breeding colony.

2. PI Responsibilities

A Principal Investigator (PI) wishing to establish a breeding colony at any TTUHSC campus facility shall submit a breeding protocol application to the IACUC using the IACUC Application Form (IAF) available in iRIS.

PI must contact the Laboratory Animal Resource Center (LARC) staff at the corresponding campus (Lubbock, Abilene or Amarillo) regarding space availability and all housing requirements needed prior to the submission of the IAF breeding protocol application.

PI shall list on the IAF application adequate numbers of personnel who are knowledgeable and experienced in breeding and who have sufficient time available to help maintain the breeding colony. The use of temporary personnel, such as summer students, is highly discouraged without direct supervision.

PI must include in the IAF a plan for reducing/avoiding/eliminating genetic drift in colonies that will be maintained for more than ten (10) generations.

After the IAF is approved, it is the PI's responsibility to work with the LARC staff to determine breeding colony accommodations in the LARC at any given time. The LARC will have control over the number of animals that can be actively housed as breeders.

PI shall breed in accordance with ***LARC Policy 010 Mouse Breeding and Colony Management*** (LARC Policy) which is based on current federal guidelines. PIs are expected to refer to the LARC policies available on the TTUHSC LARC website for further guidance and work with the veterinarians and their staff to ensure adherence. Any deviation from a LARC Policy must be approved by the Institutional Veterinarian (IVet) and the IACUC.

PI shall maintain accurate animal use records once the breeding colony has been established. The records must include the number and disposition of all animals produced. The IACUC and (IVet) strongly recommend the use of computer software programs to track of such records. The LARC staff can recommend various software programs for this task and/or advise PIs on record-keeping methods, but the ultimate responsibility for accurate records maintenance remains with the PI. All cage cards must include the approved IACUC breeding protocol number.

PI must avoid the waste of animals. US Government Principles states: *The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.* For example, there may occur periods of time when associated experimental protocols do not require a continued production of animals, yet the need for the breeding colony will recur in the future. The IACUC suggests that temporary measures, such as reduction in active breeding to the minimum number necessary to maintain the desired genotype/colony, be implemented during such periods of time.

3. Counting of Animals

In counting animals for breeding protocols, the PI must count every single animal – male and female – separately (i.e., *do not* count animals as "breeding pairs"). In preparing the breeding protocol, the PI must indicate the male/female ratio and the total number of animals that will be needed just to perform the breeding, *not* the number of animals required for associated experimental protocols (although knowledge of the number of animals required for the experimental protocol may be necessary for estimating the number of breeding animals needed).

It is recognized that animals will enter and exit the breeding program over the life of a breeding protocol. However, every animal used as a breeder in a breeding colony must be accounted for, including replacement animals that will come from the offspring. Animal numbers in the IAF should reflect the total number of animals expected to be used as breeders over the protocol's three-year period approval period, not the number of active animals at any given time.

Unweaned pups are not counted toward any protocol; only weaned animals (not the number of animals born) are counted. At weaning, all pups must be counted as either new breeding animals or as research animals for associated experimental protocols. Failure to transfer weaned animals to an experimental or breeding protocol is an IACUC violation.

4. Weaning Procedures

A. **Timing**

For rodents, all weaning must take place no later than Day 21 (see below) unless an exception is granted by the IACUC. For weaning times of other species, consult with the IVet. Failure to wean animals in a timely manner indicates inadequate monitoring/management of the breeding colony. Repeated overcrowding incidents, resulting from failure to continually assess the recommended space for use by the animals, shall be considered an IACUC violation and may result in termination of the breeding protocol.

The date of an animal's birth shall be Day 1. Therefore, Day 21 is the 20th day *after* its date of its birth. For difficult breeding colonies (i.e., animals are frequently below an appropriate weaning weight of 8–10 g), an extension to wean up to Day 28 may be requested. If such extensions are anticipated, the PI should include the contingency on the IAF application at the initial protocol review or by protocol amendment. If permission is granted by the IVet, the approval is documented.

B. **Tail Biopsy ("Snipping")**

Genetic testing of pups will be done via tail biopsy, ear punches, or other means approved by the IACUC.

Note: Per national regulatory guidance, *genetic testing of pups done via the extraction of tissue, including blood, is to be classified as, at a minimum, a Pain Category 'C' protocol, rather than as a Category 'B' breeding protocol.*

As outlined in IACUC Policy 14, tail biopsies should be done on or prior to Day 21 to avoid undue pain and distress to tested animals. Tail biopsy after Day 21 requires general anesthesia (refer to IACUC Policy 14 for further guidance and appropriate general analgesia). For tail biopsies of mice older than 21 days, scientific justification must be provided and prior approval from the IACUC must be obtained. Under many circumstances including testing after day 21, ear punches might be preferred; see Policy 14 ("Tail Snipping and Biopsy") for details.

C. **Genetic Considerations**

PI must include a plan to avoid/eliminate genetic drift from colonies, by:

1) maintaining pedigree lines and detailed colony records;
2) watching for phenotypic changes in mutants and controls;
3) refreshing breeders frequently (~every 10 generations);
4) choosing breeders at random; or,
5) other methods reviewed and approved by the IACUC.

D. **Inbred and Outbred Stocks**

In consideration of limitations of LARC resources, commercially available inbred strains, such as C57BL/6, 129, etc., may *not* be bred in-house without significant justification *and* explicit permission of the IVet.

Outbred stocks may not be bred in-house unless animals are not commercially available. If the needed outbred stocks are not commercially available and their breeding is approved by the IACUC, the PI must document and demonstrate the personnel's expertise required to correctly maintain the genetics of any outbred colonies.

E. **Disposition of Animals**
Weaned animals shall be used as follows:
1) transferred to experimental protocols;
2) used as replacement breeders;
3) eliminated from breeding protocol due to genotype or as an animal in excess of need for associated experimental protocols (euthanized by method(s) provided in the IAF breeding application).

References

1. Genotyping Procedures
2. TTUHSC IACUC Policy 10 Tail Snipping and Tail Biopsy of Rodents
3. TTUHSC LARC Policy 010 Mouse Breeding and Colony Management
4. US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training

Policy 12: Survival Surgery

1. Major and Minor Surgery

Major survival surgery is defined (*Guide*, 8th ed.) as the penetration and exposure of a body cavity, the production of substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation), or the extensive dissection or transection of tissue.

Minor survival surgery does not expose a body cavity and causes little or no physical impairment (for example, wound suturing, peripheral-vessel cannulation, routine farm-animal procedures such as castration, dehorning, and repair of prolapses, and most procedures routinely done on an "outpatient" basis in veterinary clinical practice). Minor procedures require aseptic technique and sterilized instruments as well as appropriate application of anesthesia and analgesia. Although laparoscopic procedures are often performed on an "outpatient" basis, appropriate aseptic technique is still necessary.

2. Multiple Major Surgical Procedures (General)

Multiple major survival surgical procedures on a single animal are discouraged, but may be permitted if scientifically justified by the user and approved by the IACUC.

A. Examples of acceptable justification for multiple surgeries include:
   1) the presence of related components of a research project;
   2) the conservation of scarce animal resources; and,
   3) clinical teaching purposes.

B. The principal investigator (PI) must provide clear documentation of the following items to the IACUC:
   1) the background literature, which adequately supports the need for multiple procedures and the potential significance of findings gleaned from these surgeries; and,
   2) the number of major surgeries proposed, which will be the absolute minimum required to obtain the necessary data.

3. Major USDA Species Survival Surgery

USDA Species survival surgery must be performed in the surgical suite in the local campus laboratory animal resource center, unless an exception is approved by the IACUC. Investigators must provide trained personnel to continuously observe the animal(s) until the time when the animals regain the righting reflex. Animals must be observed (and annotations made in the clinical record) for any complications that may arise for at least 5 days post-operatively (or longer if complications arise). All complications must be discussed immediately with the LARC veterinary staff. When external sutures, staples or clips are placed in an animal, they will be removed within 10-14 days of the surgery. Strict adherence to aseptic technique must be followed for all survival surgery procedures.

4. Rodent Survival Surgery

Procedures for rodent survival surgery and euthanasia are discussed in Policy 13 and Policy 21.

5. Multiple Major Surgical Procedures for Frogs

Survival surgery to harvest oocytes is described in Frog Oocyte Harvest Policy 23.

Policy 13: Rodent Survival Surgery

1. Facility

A. A dedicated facility for rodent surgery is not required. A rodent surgical area can be a room or portion of a room that is easily sanitized. The immediate surgical area must not be used for other purposes during the time of surgery.

B. Surgery must be conducted on a clean, uncluttered lab bench or table. The surface of the lab bench or table must be impervious to liquids. The work surface must be wiped with disinfectant before and after use or covered with a clean drape.

C. The surgery area MUST be separate from the area where hair is removed from the animal.

D. The area where surgery is performed MUST be a laboratory that is not currently being used for bulk storage.

2. Training

Professional and technical personnel and students who perform anesthesia, analgesia, and surgery must be trained to accomplish these tasks. The LARC Veterinarian is available to provide assistance with or training in aseptic and surgical techniques and the proper administration of anesthesia and analgesia. All new technical staff and students on protocols must complete the training provided by the LARC.

3. Instruments

A. Instrument Preparation

All instruments must be cleaned and sterilized prior to use. First, all instruments must be cleaned of any debris by hand washing or by a mechanical washer. Then, prior to surgery, the instruments must be sterilized using one of the following methods. The method of choice may be determined by the procedure, the delicacy of the surgical instruments or the devices being used. Steam autoclaving is the preferred method.

1) Heat Sterilization

a) Steam Autoclave: The instruments must be placed in a specially designed pack or wrapped in sterile drapes or cloths and secured with a thermo-sensitive tape. The use of such tape provides some indication that the autoclave procedure was effective. Instruments must be autoclaved at 121oC for 21 minutes in a vacuum autoclave. Once autoclaved, packs or wrapped instruments must be stored in closed cabinets or plastic bags. Autoclaved items must have a standard indicator to prove complete sterilization. Wrapped autoclaved items must be clearly labeled with the date of sterilization or expiration date. Items that are autoclaved in cloth wraps expire 6 months after autoclaving. Items that are autoclaved in plastic packs expire 6 months from the date of autoclaving.

b) Flash Steam: Used to sterilize articles intended to be used immediately. The temperature must reach 132oC for three to five minutes.

c) Surgical instruments should be autoclaved by LARC personnel. If research staff wish to use non-LARC autoclaves, they must have a QA program that is documented and available for review by the IVet and the IACUC. QA programs must, at minimum, consist of monthly biological indicator evaluations.

d) Autoclaved instrument packages and wrapped packs must have a date, initials, and visibly changed autoclave tape present. Autoclaved packs are good for 6 months and then must be re-autoclaved.

e) Syringes may NOT re-autoclaved for use in animals.

2) Cold (Chemical) Sterilization

Cold sterilization of instruments is not acceptable for surgical procedures. All implants (pellets, tigon tubing, catheters, etc. MUST be sterile before implantation. Generally, this means individually wrapped and sterilized when purchased. Any implant made in-house must be sterilized by either ethylene oxide or vaporized hydrogen peroxide (VHP). The LARC has the capability to perform VHP sterilization.

B Surgery on Multiple Animals
If surgeries are to be performed on a group of animals, previously sterilized instruments can be "quick" sterilized using a glass bead sterilizer or "flash" autoclaved. Instruments should be thoroughly clean of blood or tissue prior to sterilization. No more than five successive surgeries can use instruments "quick"-disinfected as described above.

1) Sterile (Hot) Bead Sterilizer: This instrument will sterilize the tips of metal instruments in 15 seconds. Instruments and glass beads should be clean and free of tissue or blood. Only clean, cooled instruments may be used on the animals. After immersion in a hot bead sterilizer, instruments should be doused in sterile saline or sterile water (in a sterile container) before use on animals to prevent thermal injury.
Note: Most sterile bead sterilizers take thirty minutes to heat.
Note: This method of sterilization may not be used for the initial sterilization of instruments; it is only appropriate when performing 5 or fewer surgeries using a single pack.

4. Anesthesia and Analgesia Selection

Contact the Institutional Veterinarian for recommendations for appropriate anesthetics and/or analgesics for the species you are using. The use of a single analgesic agent or combination will depend on the procedure performed. This table provides some guidelines for determining the expected degree of pain associated with various surgical procedures. For specific advice, please consult the Institutional Veterinarian.

SURGERY TYPE	ANALGESIC	DURATION OF TREATMENT
Skin incision	NSAID or opioid + local anesthetic	Pre-emptive + 1 dose
Open abdomen	NSAID and opioid	Pre-emptive + 48 hours
Open thorax	NSAID and opioid	Pre-emptive + 48 hours
Musculoskeletal manipulation (e.g., fracture, muscle resection)	NSAID and opioid	Pre-emptive + 48 hours
Open cranium	NSAID and opioid + local anesthetic	Pre-emptive + 48 hours
Implant or device placement (e.g., indwelling catheter)	NSAID and/or local anesthetic	Pre-emptive + 24 hours

It is important to realize that none of the analgesics provides instant relief immediately. Optimally, analgesics are provided pre-emptively to the painful event. This can be accomplished by administering the analgesics at the time of anesthetic induction. The specific analgesic choice and duration of administration to use are based on the severity of pain expected. The choices listed are not necessarily interchangeable. Please consult with the LARC Veterinarian for guidance. Analgesics may be given pre-emptively (preferred), intra-operatively to reduce inhalant requirements and provide additional analgesia, and post-operatively.

Mouse
- Buprenorphine 0.05-2.0mg/kg SQ every 6- hours SQ,
- Buprenorphine 0.05-2.0mg/kg every 6- hours SQ + Carprofen 6mg/kg q 24 hours
- Buprenorphine 0.05-2.0mg/kg every 6- hours SQ + Meloxicam 2mg/kg q 24 hours
- Extended-release buprenorphine (Ethiqa XR) 3.25 mg/kg SQ once (mouse), 0.65mg/kg (rat). Should not be given with NSAIDs.
- Carprofen 6mg/kg or SQ q24 hours ; can be combined with opioids
- Meloxicam 2.0mg/kg SQ, IP daily; can be combined with opioids
- Local: lidocaine, lidocaine/bupivacaine, lidocaine patch, bupivacaine

Rat
- Buprenorphine 0.01-0.05mg/kg SQ every 8- hours
- Buprenorphine 0.01-0.05mg/kg SQ every 8- hours + Carprofen 5mg/kg q 6-8 hours
- Buprenorphine 0.01-0.05mg/kg SQ every 8- hours + Meloxicam 1-2 mg/kg once daily
- Extended-release buprenorphine (Ethiqa XR) 0.65 mg/kg SQ once.
- Carprofen 2.5mg/kg SQ q 6-8 hours; can be combined with opioids
- Meloxicam, 1.0-2.0mg/kg SQ, daily; can be combined with opioids
- Local: lidocaine, lidocaine/bupivacaine, bupivacaine

5. Aseptic Preparation of the Animal

A. The animal must be anesthetized with a suitable anesthetic using the doses and procedure approved by the IACUC.

B. An ophthalmic lubricant must be applied to the eyes to prevent corneal drying.

C. Hair must be removed from the incision site with clippers, an appropriate razor, and/or hair removal product (i.e., Nair) applied as directed and thoroughly rinsed off to prevent continual residue action. There should be a minimum of 1cm of shaved area surrounding the incision site.

D. Skin Preparation: The bare skin at the incision site must be thoroughly cleansed with an approved surgical scrub (below) to clean the skin and create a sterile field around the incision site. Starting in the middle, and continuing in an outward spiral, apply the scrub at least three times. Wash/rinse/wipe with 70% isopropyl or ethyl alcohol, sterile water, or sterile saline after each scrub application. New gauze or applicators should be used for each cleansing. Note: Copious application of topical alcohol in rodents will soak the animal and lead to hypothermia. The use of cotton tip applicators is ideal during the skin preparation process. OB/GYN swabs with large heads work well.

The following surgical antiseptic agents (scrubs) may be used:

1) Povidone iodine scrub: A good choice for a surgical preparation with a broad spectrum of activity, including Mycobacterium. Antiseptic activity is rapid and persistent if not removed.

2) Chlorhexidine scrub: The 4% aqueous preparation effectively cleans the skin with a rapid onset of activity and a broad spectrum of activity with minimal loss of antiseptic activity.

Note: A scrub is different than a solution. A scrub contains soap and therefore has cleaning properties that a solution does not have. Scrubs are not to be mixed or diluted with water.

3) Antiseptic agents must be rinsed from the skin with sterile water, sterile saline, or 70% alcohol prior to surgery.

E. All new students and research staff must be trained by the LARC on Aseptic Technique before performing or assisting with surgeries on protocol animals.

6. Preparation by Surgeon

A. Hands must be washed with an antiseptic soap or a surgical detergent/scrub (iodophors or chlorhexidine) and rinsed with water. Sterile surgical gloves must be worn.

B. Gowns or a clean lab coat and surgical gloves are required. Sterile gloves should be donned in such a way that no skin is showing at the sleeve end. The sleeves of the lab coat/gown should not come in contact with the sterile field.

C. A new pair of sterile surgical gloves must be used for each animal. Alternatively, surgeons may wipe their gloves for 30 seconds with sterile gauze pads soaked in 70% alcohol or with chlorine dioxide for 3 minutes. Gloves must be wiped with 70% alcohol after the 3-minute chlorine dioxide application. Gloves contaminated with blood or tissue should be changed.

D. If working alone, the surgeon must have the animal anesthetized and positioned prior to gloving.

E. The first layer of a double-wrapped, sterile-packed instrument must be opened before gloving.

F. The survival surgery surgical site must be covered with a sterile drape after the surgeon has donned sterile gloves.

7.. Intraoperative Monitoring

A. The animal must be monitored carefully during the surgical procedure. Specifically, the animal's respiratory rate and characteristic response to noxious stimuli (e.g., toe pinch, and when possible, the heart rate and body temperature) should be monitored.

B. The surgical team must be trained by LARC personnel to be able to respond to the most common emergencies associated with the type of procedure being performed.

8. Post-Surgical Care

A. Post-surgical care must include observing the animal to ensure uneventful recovery from anesthesia and surgery, administering analgesics, providing adequate monitoring of the surgical incision(s), and maintaining appropriate medical records.

B. Administration of analgesia is required, except when specific IACUC approval has been granted.

C. To prevent hypothermia, place the animal(s) in a warm room or cage. To prevent suffocation, it is recommended to recover the animal in a cage with no bedding. The cage may be placed on a heat source (only ½ of the cage should be on a heating pad in order to allow the animal to move away from the heated area when ready. Heat lamps are not allowed for use with rodents.

D. Fluid replacement is recommended for all surgical procedures. Warmed sterile physiologic saline can be given subcutaneously to rodents.

E. During the recovery process, animals must be monitored continually until they regain the righting reflex.

F. Animals must be evaluated daily for at least five days by a member of the principal investigator's staff to whom post-operative care has been delegated. Animals must be monitored for evidence of excessive inflammation at the incision site, suture dehiscence (incision line failure or separation), infection, behavioral abnormalities indicative of illness (anorexia, listlessness, lethargy, dehydration, ruffled coating, lack of movement, weight loss greater than 10%). If evidence of wound infection or illness is noted, then LARC vet services must be contacted for evaluation and treatment, or the animal must be euthanized as soon as possible. Monitoring and medication of post-surgical animals must be documented on a Rodent Surgery cage notification card.

G. External sutures, staples, and wound clips must be removed 7-10 days after surgery, unless otherwise approved in the protocol or approved by the Institutional Vet.

H. If infections or other complications occur, the LARC veterinary staff must be notified immediately.

9. Surgical Records

A. A “Rodent Surgery Card” should be placed on the cage of post-surgical animals and remain until sutures/staples are removed. These cards are available from the LARC in each facility.

B. A "Surgical Record" must be completed immediately after the surgical procedure is performed. Records may be somewhat abbreviated and in composite format and can be included as part of the research data collected, but must also be available for review.

C. Records must identify the type of surgical procedure performed, the date of the procedure, the person who performed the procedure (or initials), information on all drug administration (including anesthesia and analgesia), and peri-operative monitoring, and must be maintained by the laboratory. This information must be available for review by regulatory bodies, including the IACUC.

10. Suture Selection

Surgical wounds should be closed using appropriate techniques and materials. The following table is a guide to the types of sutures available. For rodents, size 4-0 is optimal for most procedures.

Suture	**Characteristics and Frequent Uses**
Vicryl®, Dexon®	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
PDS®, Maxon®	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Prolene®	Nonabsorbable, Inert. General skin closure.
Nylon	Nonabsorbable. Inert. General skin closure.
Silk	Nonabsorbable. Excellent handling. Preferred for vascular procedures. Must not be used to suture skin.
Chromic Gut	Absorbable. Versatile material. Because gut is highly reactive to tissues, its use is discouraged.
Stainless Steel Wound Clips, Staples	Nonabsorbable. General skin closure.
Cyanoacrylate surgical glue	Generally used in addition to skin sutures or incisions less than 1 cm in length. Note that many rodents will rip the glue out, along with large areas of skin, making it difficult to close the incision. Thus, surgical glue is not recommended.

11. Exceptions

All planned deviations from this policy must be approved by the IACUC prior to the performance of the surgical procedure. Emergency situations that involve deviations from IACUC-approved procedures must be reported to the Institutional Veterinarian and the IACUC within one week of their occurrence.

Policy 14: Tail Snipping, Biopsy, Toe Clipping, and Ear Punching of Rodents

Note: Per national regulatory guidance, genetic testing done on pups via extraction of a tissue, including blood, is to be considered, at a minimum, a Pain Category 'C' protocol, rather than a simple Category 'B' breeding protocol.

1. Background

The purpose of a tail snip is to obtain a small amount of tissue and/or blood from a rodent to prepare DNA for genotyping or other analysis. The technique involves the removal of the fleshy tail tip, thus avoiding the bony vertebral segments. A tail biopsy (clipping), which is a more invasive procedure, involves the amputation of the tail between bony vertebral segments.

Both tail procedures usually lead to bleeding at the site. A tail snip is easily performed on mice ≤ 21 Days. When ≤ 2 mm length of tissue is collected from these young mice, the procedure does not cause the animal undue pain or distress because the vertebra in the distal tail tip are not yet calcified.[1, 2] By analogy, this procedure is similar to human ear piercing, which does not require the use of anesthetics nor analgesics. When a similar procedure is performed on older mice (> 21 Days), or when > 2 mm length tissue is collected, this constitutes a tail biopsy (clipping) because the tissue removed will likely contain calcified vertebrae.[1, 2] A tail biopsy (clipping) is considered to be a painful procedure and requires the use of anesthetics and analgesics, unless scientifically justified and approved by the IACUC.

Ear punching is a technique used for both identification of an animal and genotyping.

Toe clipping is the practice of removing the toe from the most distal joint to the tip of the toe. Toe clipping is not necessarily considered a surgical procedure.

The IACUC discourages the practice of tail biopsy (clipping) and toe clipping for the purpose of animal identification but acknowledges that under certain circumstances it may be necessary. These procedures are regarded as potentially more than momentarily painful and, therefore, the IACUC has determined that these procedures shall not be performed on laboratory rodents without adequate justification and prior approval. As a method of identification of small rodents, toe-clipping should be used only when no other individual identification method is feasible - although it may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age, especially if toe clipping and genotyping can be combined.[3,4] Under all circumstances aseptic practices should be followed. Use of anesthesia or analgesia should be commensurate with the age of the animals.[2]

2. Guidelines for Tail Snips (collecting ≤ 2 mm tissue from mice ≤ 21 Days)

A. A tail snip in this age period is easy to perform and does not cause undue pain or distress. The use of anesthesia is suggested but not required.

B. Tail snipping should be performed using sharp scalpel blades, scissors or razor blades. The length of tissue collected should not exceed 2mm.

C. If tail snips are performed on multiple animals using the same scalpel or razor blade, the use of each blade should be limited to five (5) animals/blade. (Be aware that cross-contamination of DNA samples may occur when using the same scalpel or razor blade on multiple animals. Cleaning the instrument between use on each animal with 70% ethanol is often used to prevent contamination.).

D. Bleeding from the sampling site may stop spontaneously, but if it does not stop, adequate hemostasis may be achieved by applying brief pressure on the stump with gauze or a cotton ball, and/or application of a cautery pen, styptic pen or silver nitrate to the site.

E. In most cases, the IACUC recommends that tail snipping for genotyping be performed on animals that are 10-14 days old. If genotyping must be performed after weaning, the weaned animals must be moved to an IACUC-approved experimental protocol.

3. Requirements Specific for Tail Biopsy (Clipping) (mice > 21 Days and/or > 2 mm tissue collection)

A. Removal of tail segments including amputation between bony segments is considered to be a painful procedure and requires general anesthesia and analgesics as studies support that tail biopsy in older ages and/or of greater length may result in multi-week effects on behavior and physiology.[7] Therefore, if anesthesia and/or analgesics are contraindicated, the investigator must provide adequate scientific justification and obtain prior IACUC approval.

B. Alternatives to tail snips and biopsies should be considered. Small quantities of blood from distal veins (e.g., saphenous vein) or skin samples from ear punches may be used for analysis, and PCR assays using cheek swabs and hair bulbs have also been described[3-6].

4. Guidelines for Ear Punching for genotyping and Identification

A. Method that removes small pieces of tissue using an ear punch device. Ear punch or notching instrument is disinfected with alcohol or a hot bead sterilizer between animals to avoid sample contamination

B. Procedure should be performed on animals (> 14 d) when the pinnae (ears) are generally large and thin enough to punch/notch as a method of identification as well as to obtain tissue for genotyping.

C. Ear punching does not require anesthesia.

D. Mouse Ear Punching (see Fig. 1a for examples)

1) The mouse is restrained by the scruff (see Fig. 1b) and an ear puncher is used to make holes and/or notches in the ears following an identification chart.

2) Hemostasis of the ear punch/notch site can be achieved by compression.

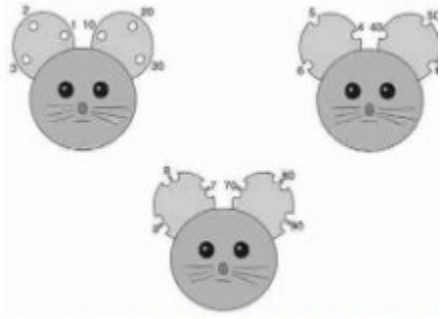

Fig. 1a Mouse ear punch

Fig. 1b Mouse restraint

E. Rat Ear Punching

1) Firmly restrain the animal. Restraint methods include:

- The two-handed technique: First place the rat on your arm, holding the base of the tail with your hand. Hug the rat at the shoulders and push in gently at the elbows to cross the front legs. Still maintaining your hold at the base of the tail, stretch out the abdomen. Grasp the rear legs and immobilize (see Fig. 2a).
- If more restraint is needed, the rat may be scruffed by grasping the loose skin behind the neck.

- A restraining device, which allows access to the head while still immobilizing the animal, may also be used. An ear puncher is then used to make holes and/or notches in the ears (a second person will be needed to do this).

2) Ear puncher (see Fig. 2b) is used to make holes and/or notches in the ears following an identification chart.

3) Hemostasis of the ear notch/punch site can be achieved by compression. Ear punch or notching instrument is disinfected with alcohol or a hot bead sterilizer between animals to avoid sample contamination

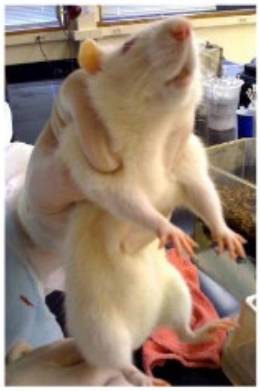

Fig. 2a Rat restraint

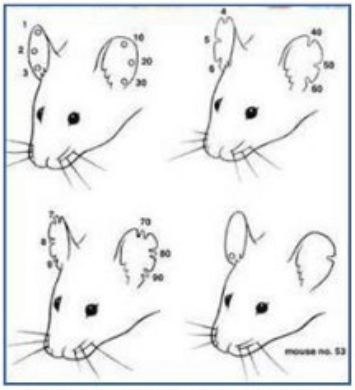

Fig. 2b Rat ear punches

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4. Schaefer DC, Asner IN, Seifert B, Bürki K, Cinelli P. 2010. Analysis of physiological and behavioral parameters in mice after toe clipping as newborns. Lab Anim 44:7-13.

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2. Guide for the Care and Use of Laboratory Animals, 8th Ed., National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US); 2011. ISBN-13: 978-0-309-15400-0 ISBN-10: 0-309-15400-6

Policy 15: Endpoints regarding Severe or Chronic Pain or Distress

This policy provides a guideline of default endpoints for animal research studies, for use by Principal Investigators (PIs) and project personnel, when either anticipated or unanticipated, severe or chronic pain or distress occurs. The PI should identify and justify alternative endpoints in a protocol application. The default endpoints below can be used for protocols that have the potential for pain and distress. If an animal research study involves death as an endpoint, the PI shall include the information required by Section 3 below in his/her protocol application.

Definitions

Experimental Endpoint: This occurs when the scientific aims and objectives have been reached.
Humane Endpoint: The point at which pain and distress is prevented, terminated, or relieved in an experimental animal.
Moribund: The point at which an animal is in the state of dying. This is frequently seen as an inability to right; hypothermia, unresponsive, unable to walk or some combination thereof.
Death as an Endpoint: Study that requires an animal to die without benefit of intervention or humane euthanasia.

1. Humane Endpoints

Humane endpoints are those endpoints that will remove an animal from a study before the experimental endpoint has been reached. Humane endpoints refer to one or more predetermined physiological or behavioral signs that define the point at which an experimental animal's pain and/or distress is terminated, minimized or reduced by taking actions such as euthanizing the animal, terminating a painful procedure or giving treatment to relieve pain and/or distress. Humane endpoints function as an alternative to death as an endpoint and provide investigators with an effective way to refine their research. The establishment of humane endpoints prior to the start of an experiment allows the investigator to prevent unnecessary animal pain and distress while ensuring accurate and timely data collection.

To be effective, humane endpoints must be clearly defined and based on objective criteria. Non-specific signs of illness such as inactivity, hunched posture or a rough coat are an indication that an animal should be examined more closely. Familiarity with the animal model in use is necessary to select endpoints that are both humane and scientifically sound. As experience with and data collected from a specific animal model accrue, endpoints can be refined or modified. Humane endpoints should be specific to the condition being studied. For examples, see the scoring sheets at the end. While every study does not need a scoring sheet, you can see how these sheets reflect possible clinical conditions and are relevant to the condition being studied.

2. Default Endpoints

Unless a PI identifies and adequately justifies alternative endpoints and the IACUC approves them, endpoints for laboratory animals, including, but not limited to, nonhuman primates, dogs, cats, pigs, sheep, goats, rabbits and rodents, will be triggered by any of the following conditions:

1. Loss of 20% of body weight from baseline weight when assigned to the protocol. A growth nomogram must be used to adjust the basal weight for growing animals.
2. Organ failure or major medical conditions that are unresponsive to treatment such as respiratory distress, icterus (jaundice), uremia (loss of renal function), intractable diarrhea, self-mutilation or persistent vomiting.
3. Surgical complications that are unresponsive to immediate intervention; i.e. bleeding, vascular graft/circulation failure, infection, and wound dehiscence (rupture of sutures).
4. Rodents that have complete anorexia for 2 days or non-rodents that display anorexia for 4 days.
5. Other clinical or behavioral signs in rodents or non-rodents that are unresponsive to appropriate intervention. In the case of rodents, these are defined as abnormalities persisting for 24 hours and for non-rodents, for 48 hours.

Abnormalities would include:

a. inactivity
b. labored breathing
c. sunken eyes
d. hunched posture
e. piloerection/matted fur
f. one or more unresolving skin ulcers
g. abnormal vocalization when handled
h. tumors that affect normal function or that become ulcerated
i. persistent coughing
j. excessive scratching or inability to rest due to dermal changes

The circumstances described above represent a conservative minimum and are not necessarily consistent with pain-and-distress-free research. In his/her protocol applications, the PI must identify endpoints that avoid or minimize discomfort, distress and pain to the animals and that are compatible with experimental objectives.

Appendix 1 and 2 provide examples of alterative assessments that may be used to establish humane endpoints in specific IACUC protocols, depending on how the experimental model affects animal physiology.

If the LARC or laboratory staff identify an animal that displays any of the behaviors described above, the LARC or laboratory staff shall immediately report their observations to the PI and the Institutional Veterinarian (IVet). If the PI identifies an animal as having any of the behaviors described above, he/she shall immediately follow this policy and euthanize the animals, unless an exception to these criteria has been approved in the IACUC protocol. If an animal has any of the endpoints identified above and the PI feels that the animal should not be euthanized, the IVet should be consulted immediately. In this circumstance the IVet will make the final, clinical decision regarding the need to euthanize the animals.

3. Institutional Endpoints

The IACUC upholds statements put forth by the PHS Policy, AWA Regulations, and The Guide, regarding the care and use of animals, especially those that may experience pain and distress. Animals must not be allowed to suffer beyond the point where justifiable scientific objectives have been achieved. The IACUC requires very thoughtful scientific rationale and planning for the use of death as an endpoint in studies that cause morbidity, moribundity and mortality. If the protocol involves death as an endpoint, the following shall be included:

Scientific Justification:

1. What specific set of circumstances requires death, what alternatives were considered, and how alternatives will be used whenever possible.
2. Why efforts to relieve pain and/or distress cannot be used.
3. Why the number of animals proposed is the minimum necessary to achieve scientific objectives.
4. What information will be gained by allowing death as an endpoint.
5. Has the LARC veterinarian been consulted on alternatives to death as an endpoint? If not, please do so.
6. Explain why morbidity as an endpoint cannot be used.

Plan:

1. Personnel are trained to recognize signs of morbidity and moribundity of animals on study.
2. Written monitoring records are kept current and available to veterinary staff and IACUC.
3. Monitoring of animals increases with level of morbidity and does NOT stop during weekends or holidays.
4. The IVet is promptly notified when animals show signs of morbidity and moribundity that were unexpected, or of greater intensity or duration than those described in the animal protocol.

References

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2. Canadian Council on Animal Care, "Guidelines on: Choosing an appropriate endpoint in experiments using animals for research, teaching and testing".http://www.ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf

3. Toth. "Defining the Moribund Condition as an Experimental Endpoint for Animal Research." *ILAR Journal*. 41(2) January, 2000, p. 72.

Policy 15 Appendix I. Example Scoring Systems for Humane Endpoints

The following system parameters should be assessed in the order listed in the table. Evaluation of behavior and neurologic signs requires minor handling. Hydration and weight loss require manipulation of the mice. Care should be taken when manipulating animals with significant compromise.

Clinical scoring system for tumor implantation mice (cranial injection)

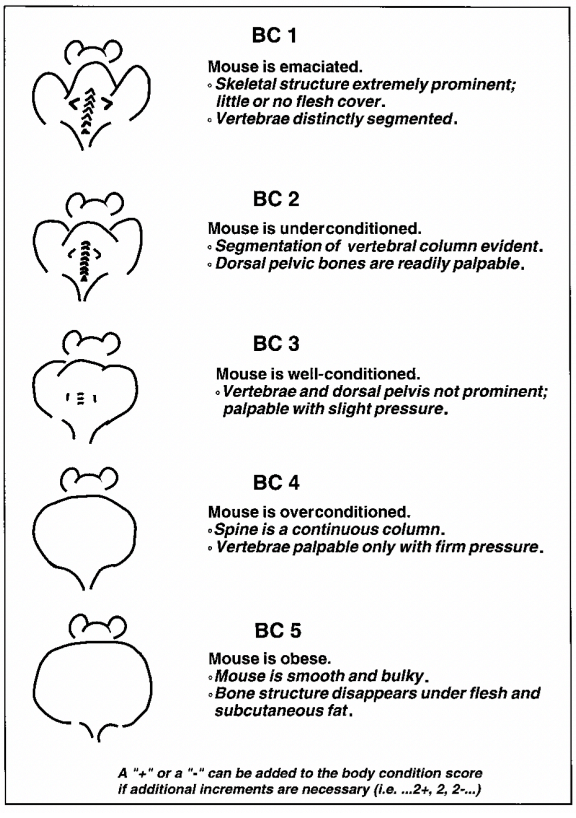
Scoring:

a. **Reluctant to move**—mouse is easily restrained from the cage floor. Reduced flee response when gloved hand is placed around the mouse.
b. **Delayed righting response**—the righting response assesses both motor coordination and vestibular function. The mouse is placed in a supine position and the time taken to right (all four limbs placed under the body) is assessed. Normal mice will immediately turn over. Delay to righting of greater than2 sec should be scored as 3.
c. **Decreased grip strength**—mouse is held at the base of the tail, lowered towards the cage hopper and allowed to grasp the wire bars. The mouse is gently pulled backwards to test ability to maintain grip on the wire bar. Abnormal mice do not grasp the bars or are easily displaced (weak).
d. **Clasp response**—The mouse is suspended by the base of the tail. Normal mice will elevate the head and extend the limbs in an attempt to place the feet (especially when lowered towards a surface). Abnormal mice clasp the forelimbs, pelvic limbs or both.
e. **Cranial deformity**—for tumors that result in deformity of the cranium, the distance between the bases of the ears is measured. Significant enlargement in the vertical plane will also be considered an endpoint.

Interventions:

HEP = humane endpoint (euthanasia required)
0-2 = Normal, monitor daily
3-6 = Monitor twice daily, contact veterinarian for treatment options
>6 = Euthanasia
If category score for any one category is 3 or more, contact veterinarian for treatment options.

Policy 15 Appendix 2.
Body Condition Scoring for demonstration purposes
(Ullere-Cullere, MH, *Laboratory Animal Science*, **49**, 319 (1999)



Policy 16: Use of Non-Pharmaceutical Grade Compounds

1. Background

For teaching or research that involve animals, investigators are expected to use pharmaceutical-grade compounds and vehicles whenever they are available, even for acute and/or terminal procedures. A pharmaceutical-grade substance is any active or inactive drug, biologic, reagent, etc., manufactured under Good Manufacturing Practices (GMP) which is approved, conditionally approved, or indexed by the Food and Drug Administration (FDA) or for which a chemical purity standard has been written or established by a recognized compendia (e.g., United States Pharmacopeia-National Formulary [USP-NF], British Pharmacopeia [BP])[1]

Issues such as sterility, pyrogenicity, stability, pharmacokinetics, and quality control have usually been addressed during the course of producing pharmaceutical-grade drugs. the same cannot be for substances produced in the research laboratory. The use of non-pharmaceutical-grade chemical compounds in experimental animals is acceptable if particular conditions are met.

2. Procedure to obtain IACUC Approval

Non-pharmaceutical-grade chemical compounds (including, but not limited to, expired pharmaceutical drugs) is acceptable in research or teaching when animal use is required, if reviewed and approved by the IACUC *prior* to the first use of the compound. The following circumstances must be presented for consideration by the IACUC:

A. Scientific necessity
B. Lack of availability of an acceptable veterinary or human pharmaceutical-grade compound

Per OLAW[1], IACUC considerations for the use of non-pharmaceutical-grade chemicals may include:

- grade
- purity
- sterility
- acid-base balance
- pyrogenicity (endotoxins)
- osmolality
- stability
- site and route of administration
- compatibility of components
- side effects and adverse reactions
- storage
- pharmacokinetics

Note: Cost savings alone do not adequately justify the use of non-pharmaceutical-grade compounds in animals.

In preparing proposals that will incorporate the use of non-pharmaceutical-grade chemical compounds, investigators should address the quality of the preparations (i.e., issues of purity, stability, and sterility (to include endotoxins). Investigators should explain to what extent purity and sterility will be maintained in the preparation and administration of the compound, especially when administered parenterally. In addition, the methods and routes of administration of the compound must be described. Information about stability and pharmacokinetics should be given when available.

References

1. OLAW FAQs Section F.4
2. Animal Care Policy Manual. Policy #3 Veterinary Care
3. NIH Policy & Compliance. OLAW FAQs about the PHS Policy on Humane Care and Use of Laboratory Animals

Policy 17: Expired Drugs

1. Background

The use of expired drugs or medical materials (i.e., fluids, disinfectant solutions, catheters, sutures) in animals is considered both inadequate veterinary care and poor experimental technique. These materials may lose potency, function, or even degrade to toxic byproducts if stored after their expiration dates resulting in unpredictable effects that can jeopardize animal welfare and affect experimental results. Even pharmaceutical grade drugs are subject to these effects..

2. Responsibility

Each researcher is responsible and accountable for ensuring that expired materials are not used in animal research. Principal Investigators (PIs) and laboratory staff are responsible for ensuring that expired drugs and medical materials are properly disposed after their expiration date or labelled "Not for Use in Animals".

3. Protocol Procedures

A. **Drug:** for this purpose, any regulatory agency approved or investigational substance, agent, biologic, or chemical listed in a pharmacopeia, chemical supply catalogue, or synthesized or isolated extemporaneously in a laboratory, and administered to an animal by any route, including injection, inhalation, topical application, ingestion, electroporation or suppository, for use in the investigation, diagnosis, cure, mitigation, treatment or prevention of disease or biology in humans or animals.

No expired drugs or fluids are allowed for use on animals in research or instruction, including terminal procedures. All drugs must be discarded within one month of the manufacturer's expiration date or labelled "Not for Use in Animals".

B. **Expiration Date:** All chemicals used on or in animals must have an expiration date clearly labeled on the container. The expiration date is the date printed on the label/package for materials with a manufacturer's expiration. While it is understood that manufacturer expiration dates are often conservative, nonetheless, this represents the most practical approach to the issue.

C. **Drugs without expiration dates should be dated when opened:** The investigator should determine the stability of the drug to identify a reasonable shelf-life. This is commonly obtained from the manufacturer. If stability is unknown, the drug should not be used beyond one year.

D. **Dilutions, Reconstitutions, Compounding:** For drug preparations made by the investigators, the expiration date is no more than thirty (30) days from the date of preparation. Such materials should be prepared using aseptic (sterile) technique, under proper storage conditions, and should be labeled by name, drug concentration, and the new expiration date as soon as they are prepared. The expiration date may vary from the manufacturer's expiration date. If drug or material is transferred to another container, it should be clearly labeled with the name of the drug or material and the expiration date of the original stock. An item is considered expired the day after the month or date indicated on the label (i.e., an item labeled January 2021 would be considered expired on February 1, 2021). When investigators wish to access sterile diluents multiple times (i.e. to obtain small volumes for administration and drug mixing), the investigators can do so only if they do not add any chemical to the fluid, they access the fluid(s) aseptically and they store the fluid(s) as recommended by the manufacturer. Under these conditions, the investigator can use the sterile fluid(s) for up to thirty (30) days after initial opening.

E. **Medical Material:** A non-bioactive substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease or a substance (other than food) intended to affect the structure or any function of the body. Examples include but are not limited to: gauze, sutures, catheters, etc.

Medical materials such as catheters, surgical instruments, sterile gloves, suture materials, and pressure transducers are often labeled with an expiration date to assure sterility. In general, after the expiration date these materials must be discarded. Expired materials capable of being re-sterilized should be dated with a new expiration date appropriate for the package being used. If the PI obtains information from the manufacturer that provides documentation that a particular material or instrument has been shown to remain sterile beyond the date on the packaging, a request to use the item in survival studies will be considered by the IACUC.

F. **Exceptions:** Under special conditions, an investigator might require use of an expired or non-pharmaceutical grade substance (for example, no pharmaceutical-grade drug is available, or a pharmaceutical-grade drug is available, but is not compatible with the delivery requirements of the protocol). To do so, the investigator must provide scientific justification for such usage in her/his animal protocol, and the protocol must be approved by the IACUC or, in a nonroutine urgent situation, by the IVET in writing.

4. IACUC Recommendations

A. The IACUC recommends that each laboratory establish an inventory procedure to facilitate the identification and discarding of expired drugs.

All medical materials and supplies used in live animals, including acute procedures, should be individually labeled. The LARC area supervisor in LARC shared procedures rooms, or the Principal Investigator for all other shared-use laboratories, shall be authorized to discard materials which cannot be clearly identified by owner, whether or not they have reached their expiration date. EH&S will be contacted to arrange for disposal of unidentifiable contents.

The following are general guidelines suggested to help comply with this policy:

- Store all drugs in one location (to make checking easier). Please contact LARC if you would like assistance in obtaining a lockable box for your drugs.
- Consider assigning the inventory responsibilities to one specific individual, with another individual assigned as backup.
- Establish an inventory system which minimizes the amount of drug or medical supplies on hand.
- Perform regular monthly checks of your inventory and discard all expired drugs or medical materials.[4]
- Contact your suppliers to see if they will accept the return of some expired drugs or medical supplies for credit.
- Place all expired drugs and medical materials in a clearly labeled container while they await pickup for disposal or return to manufacturer.

5. IACUC Regulations

Agents that provide anesthesia and analgesia must be used before their expiration dates and should be acquired, stored, their use recorded, and disposed of legally and safely.[1,4]

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...[2]

The use of expired medical materials (e.g. drugs, fluids, sutures, anesthetics, sedatives, or analgesics) during any survival surgical procedure on a regulated species is not considered acceptable veterinary practice and therefore not consistent with adequate veterinary care as required by the regulations promulgated under the Animal Welfare Act.[3] The finding of expired drugs and/or materials during a USDA inspection may result in a citation for inadequate veterinary care.

References

1. Guide for the Care and Use of Laboratory Animals, Eighth Edition, p. 123.
2. PHS Policy on Humane Care and Use of Laboratory Animals, FAQ F.5.
3. USDA Animal Care Resource Guide Policy 3.
4. TTUHSC OP 73.04 Research Involving Controlled Substances and Laboratory Apparatus

Policy 18: Experimental Neoplasia in Rodents

1. Background

Experimental induction of neoplasia presents concerns for animal welfare. In particular, the humane endpoint for the animal bearing the tumor must be clearly described and that endpoint must be approved by the IACUC prior to the initiation of any procedures.

Proposals that involve experimental neoplasia usually involve three types of studies, including those that 1) increase our understanding of biological mechanisms, 2) aid in the design of efficacious treatments, and 3) facilitate production of antibodies via ascites production.

A. The first type describes studies of how cancer cells grow and behave. This policy is intended to limit the tumor burden to that which does not cause excessive pain or distress, but achieves the research goal.

B. The second type involves studies of the response of neoplasms to chemical, radiologic, or immunologic interventional therapy. In this class of study, not only must the tumor burden be considered, but possible deleterious side effects/toxicities of the treatment must also be evaluated.

C. The third type of study involves the production of experimental reagents (antibodies) by the injection of cell lines that retain properties of cancer cells. In this special case, the goal is to limit the volume of ascites liquid to that which does not interfere excessively with normal function of the animal.

2. Consideration of Alternatives

Outcomes of tumor studies vary depending on the species and strain of animals used, the route of administration used for the growth of transplantable tumors and the subsequent modality employed in cancer treatment studies. It is up to the investigator to determine whether alternatives to using live animals are available and are appropriate for their study. It is very rare that "death as an endpoint" studies (i.e., survival studies) will be allowed by the IACUC. In considering such studies, the PI must examine all possible alternatives and present evidence to the IACUC that none are scientifically acceptable for the proposed outcome.

Note: Citing other studies in which 'death as an endpoint' has also been used is NOT a scientific justification.

3. Procedural Guidelines

A. General Guidelines

<u>Pathogen Testing</u>

Because transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine or human viruses that can contaminate rodents, all transplantable murine tumor sources must be assayed for contamination with adventitious murine and human viruses prior to xenografting to prevent the possible spread of pathogens into the rodent colonies and personnel. If tumors are derived from human sources, the requirement for Institutional Review Board (IRB) approval should be discussed; human cell lines and/or tissues must be registered with the Institutional Biosafety Committee (IBC); and, laboratory members must complete the Bloodborne Pathogen Training. If pathogen testing has not been done on the material, contact the Institutional Veterinarian (IVet) for recommendations and outsourced contracting options.

<u>Tumor Xenografts</u>

All protocols involving experimental neoplasia in rodents must be consistent with IACUC Policy 15: Endpoints regarding Severe or Chronic Pain or Distress. The Guide (2011) defines the humane endpoint as "the point at which pain or distress is prevented, terminated or relieved in an experimental animal." In addition to the Default Endpoints listed in IACUC Policy 15, the percentage of tumor mass to body weight and the animal's general well-being must be considered for those superficial tumors that can be monitored by palpation and measurement. Unless exceptions can be scientifically justified and are approved in advance by IVet and IACUC, the following guidelines must be followed and euthanasia is required when:

1) Solid tumors, subcutaneous (s.q.)
   a) Calculate the mass of each tumor mass using the following formula:
      - Mass = tumor volume (mm^3) = 0.5 x length (L) x width (W) x height (H) measured using calipers (1)
      - Add the volume of multiple tumors together.
      - For mice, no single tumor may exceed 2 cm^3; the total tumor volume may not exceed 3.4 cm^3.
      - For rats, no single tumor may exceed 16 cm^3; the total tumor volume may not exceed 27 cm^3.
   b) The animal is unable to drink, eat, ambulate, defecate or urinate due to a tumor burden.
   c) The tumor is ulcerated and/or infected.

2) Solid tumors, orthotopic, metastatic, etc.
   Tumors induced in body cavities (ex: cranium, orbit, abdomen, thorax) or organs may be more difficult to monitor for progression and may have additional limitations as to the maximum acceptable size or duration. These animals must be monitored closely for any severe impairment in physiological or neurological function and be euthanized as soon as such signs become apparent. Humane endpoints pertinent to the tumor model must be defined in the protocol. For example, with brain tumors, the endpoints must reflect neurological deficits and/or cranial deformity.

3) Hematological tumors
   Hematological cancers (ex: leukemias) may be more difficult to monitor for progression. These animals must be monitored closely for any severe impairment in physiological or neurological function and be euthanized as soon as such signs become apparent. Humane endpoints pertinent to the model must be given in the protocol.

4) Myelomas and Ascites Production
   After inoculation with an ascites-producing tumor cell line, animals must be observed at least three times per week for the first week and daily thereafter to monitor the degree of abdominal distention and signs of illness. Ascites fluid should be removed by peritoneal tap before abdominal distention is great enough to cause discomfort or interference with normal activity. Animals should be euthanized if they become moribund (i.e., huddling, hunched posture, increased respiratory rate and/or effort, lethargy, difficulty with normal ambulation, or ruffled coat). Animals should be tapped before they have gained 20% of their baseline body weight. Three abdominal taps, with the last tap being terminal, is permitted.

B. Interventions
   After the study has begun, any deviation from the default endpoints (including death as an endpoint), must be reported immediately to the LARC veterinary staff for clinical evaluation. For example, if the tumor severely impairs normal bodily functions or the animal appears to be in distress, the veterinarian will prescribe treatment/monitoring that may include humane euthanasia.

C. Pain category assignment
   Assignment of pain category by the IACUC for studies involving tumor-bearing animals shall be in accordance with Policy 5: Pain Categories for Experimental Protocols. However, because each study and each tumor line is unique, the IACUC will review each protocol individually and consider circumstances that may impact the assignment of the appropriate pain category. In the event that the procedure being proposed will cause pain or distress and analgesics cannot be administered for scientifically justified reasons, the PI must describe additional methods for ensuring that discomfort, distress, and pain will be limited to that which is unavoidable in the conduct of this project.

References

1. Guidelines for the welfare and use of animals in cancer research. Workman, P., et al. (2010). *Br J Cancer, 102*(11), 1555-1577. doi: 10.1038/sj.bjc.6605642
2. Determination of subcutaneous tumor size in athymic (nude) mice. Tomayko, M.M. and Reynolds, C.P. (1989). *Cancer Chemother Pharmacol, 24*(3):148-54. doi: 10.1007/BF00300234

Policy 19: Use of Freund's Complete Adjuvant and Other Adjuvants

1. Background

Adjuvants are compounds that stimulate the immune response. Although adjuvants (particularly Freund's Complete Adjuvant (FCA)) are useful and sometimes essential for producing antibodies, they are capable of causing severe inflammation. FCA is a water-in-oil emulsion containing either killed *Mycobacterium butyricum* or *Mycobacterium tuberculosis*, and is used to enhance antigenicity and stimulate an immune response greater than the antigen alone.

In order to reduce the amount of inflammation-induced distress in research animals, Principal Investigators (PIs) must consider using alternative systems that reduce the number of animals used (e.g., tissue culture, chicken eggs, etc.), as well as the use of non-inflammatory adjuvants (e.g., Sigma Adjuvant System (SAS), Freund's Incomplete Adjuvant, TiterMax, muramyl dipeptide, ethylene-vinyl acetate copolymer).

This policy establishes reasonable guidelines for the use of FCA and other adjuvants which minimize the associated pain and distress due to undesirable side effects. Principal investigators (PIs) should be aware that animal welfare regulations and policies are constantly changing and this policy will be revised as needed. Deviations from the recommendations in this policy must be scientifically justified in the protocol and approved by the IACUC prior to their use.

2. Guidelines for the Use of FCA and Other Adjuvants

A. The use of FCA is highly discouraged, as it is recognized to cause more than momentary or slight pain[1]. It is expected that the Investigators will try another adjuvant before using FCA. The use of FCA or other adjuvants must be scientifically justified in an IACUC protocol and approved by the IACUC prior to their use. The protocol must include:
   1) the identification of the antigen
   2) the adjuvant or solution used for injections
   3) the volume delivered per injection site
   4) the total volume to be injected
   5) the site of injection
   6) the boosting schedule
   7) the justification for the number of animals exposed to the adjuvant
   8) scientific justification for the use of FCA

B. The investigator should strongly consider the administration of analgesics with use of either complete or incomplete Freund's adjuvant. Analgesics will provide relief from any pain or distress caused by the adjuvants.

C. The use of FCA must be limited to the initial immunization. Animals will be monitored daily for adverse effects and consult with the Veterinarian should they occur. Booster injection solutions must contain incomplete Freund's adjuvant, saline solution, or other suitable booster reagent.

D. Depending on the species being used (see the table below), FCA-containing injections may be given by either subcutaneous, intramuscular, or intraperitoneal routes. Intravenous injections can damage the lungs by creating a lipid embolism and shall not be used.
   1) Footpad injections in rabbits are not allowed.
   2) Footpad injections in mice are strongly discouraged (exceptions may be made for mice if scientifically justified, and must fulfill the following criteria:
      a) only one injection per mouse will be allowed.
      b) they must be housed on soft bedding.
      c) they must be monitored daily for potential health problems.
      d) euthanasia must be performed for mice that exhibit signs of severe pain or distress, including (but not limited to) persistent inactivity, labored breathing, sunken eyes, hunched posture, piloerection/matted fur, unresolving skin ulcers, abnormal vocalizations when handled, anorexia, or excessive scratching.

E. The number of injection sites and injection volumes will vary depending on the type and size of the animal used and the route of administration applied. Maximum allowed volumes per injection site and total volumes administered at each immunization are listed below for representative species. Smaller volumes may be equally effective. Multiple injection sites must be separated from each other by enough distance to maintain an adequate blood supply to the area.

Routes of Administration				
Species	Subcutaneous	Intramuscular	Intraperitoneal	Footpad
Mice	0.1/0.1	NR*	0.25/0.25	0.01-0.05
Rats & Hamsters	0.1/0.4	0.1/0.2	0.5/0.5	0.10
Rabbits & Guinea Pigs	0.1/1.0	0.25/0.5	NR*	Not allowed
Goats	0.2/1.0	0.25/1.0	NR*	Not allowed

* Not recommended

F. In order to prevent infection at the site of the injection and to observe the injection site for complications, aseptic preparation of the site is required. This includes clipping of the fur and the use of a skin disinfectant.

G. Some antigen-FCA combinations will result in open draining skin lesions even when used at recommended dosages. If this occurs, the lesions must be treated appropriately to prevent infection.

H. Preferred injection sites are along the back and sides of the animal; these areas are generally not used in handling or restraining the animal and are easily visible for observation and allow the best lesion drainage should lesions occur.

References

1. USDA/APHIS *Animal Care Policy Manual* (2017, Oct 1) *Policy 11 Painful and Distressful Procedures* retrieved from (https://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf)

Policy 20: Cervical Dislocation or Decapitation of Animals

The recommendations of the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition serve as the standard for acceptable methods on euthanasia.

1. Euthanasia by Cervical Dislocation

The IACUC will allow cervical dislocation to be used as a primary method for euthanasia only for mice and rats (under 200g body weight), and only after demonstration by appropriate lab members of proficiency in the technique. (Please see Policy 21: Euthanasia for a description of the use of cervical dislocation or decapitation to confirm death after inhalant overdose.) Per AVMA 2020 Guidelines, Section M3.6, Cervical Dislocation: "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 the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull… Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation in rats..." In most cases the IACUC will require anesthesia or sedation prior to cervical dislocation, and bypassing this requirement must be justified and approved.

In heavier animals (e.g., rats over 200g body weight and rabbits over 1 kg body weight), the greater muscle mass in the cervical region makes manual cervical dislocation physically more difficult. Therefore, other methods of euthanasia must be performed on these types of animals. Please see IACUC Policy 21: Euthanasia.

It is the PI's responsibility to determine that all personnel have been trained to perform the technique of manual cervical dislocation, and to monitor that personnel consistently apply it humanely and effectively.

2. Euthanasia by Decapitation

The IACUC generally discourages the practice of euthanasia by decapitation, but recognizes that for some studies this method may be necessary. Decapitation must be performed by trained personnel, in a safe manner, and using sharp instruments.

The PI is responsible for maintaining their decapitation apparatus in good working order, including maintenance of blade sharpness. The use of plastic cones to restrain unanesthetized animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal for decapitation. Therefore, the use of plastic cones prior to decapitation is strongly encouraged.

It is also the PI's responsibility to determine that all personnel have been trained to perform the technique of decapitation, and to monitor that personnel consistently apply it humanely and effectively.

A. Rodents: Euthanasia by decapitation should normally be performed while the animal is under general anesthesia and may be used in research settings only when its use is required by the experimental design and is approved by the IACUC. Per AVMA 2020 Guidelines, Section M3.7, Decapitation: "Although it has been demonstrated that electrical activity in the rodent brain persists for 13 to 14 seconds following decapitation[1], more recent studies indicate that this activity does not infer the ability to perceive pain, and conclude that loss of consciousness develops rapidly[2]." Therefore, decapitation of rodents and small rabbits (<1 kg) is conditionally acceptable if performed correctly by trained personnel. For age-specific guidelines, please see IACUC Policy 21: Euthanasia.

B. Amphibians, Fish and Reptiles: The central nervous system of amphibians, fish, and reptiles is tolerant to hypoxic and hypotensive conditions[3]. Therefore, decapitation of these species should be performed under anesthesia and must be followed immediately by pithing. Please see IACUC Policy 21: Euthanasia.

References

1. Cooper JE, Ewbank R, Platt C, et al. Euthanasia of amphibians and reptiles. London: UFAW/WSPA, 1989.
2. Holson RR. Euthanasia by decapitation: evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. Neurotoxicol Teratol 1992; 14:253-257.
3. Vanderwolf CH, Buzak DP, Cain RK, et al. Neocortical and hippocampal electrical activity following decapitation in the rat. Brain Research 1988; 451:340-344.
4. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Policy 21: Euthanasia

Euthanasia (from the Greek, meaning "good death") is a critical component of humane animal care. In general, the recommendations of the AVMA Guidelines on Euthanasia (2020) serve as the standard for acceptable methods on euthanasia.

Carbon dioxide inhalation is the preferred method of rodent euthanasia. Intraperitoneal barbiturate overdose and isoflurane overdose (with secondary procedure) may be used in certain circumstances. Any other method of euthanasia requires justification in the Animal Protocol.

Unless overwise noted, non-rodent species (under USDA jurisdiction) must be euthanized by intravenous barbiturate overdose. Principal Investigators should consult with the Institutional Veterinarian in these cases.

The following guidelines provide important criteria for the successful implementation of inhalant anesthesia.

- Animals must be euthanized only by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements.
- Death must be induced as painlessly and quickly as possible.
- Upon completion of the euthanasia procedure, death must be confirmed by a secondary method as noted below.
- Euthanasia may not be performed in the animal room.
- The euthanasia method must be appropriate to the species, approved in the animal study proposal, and conform to the most recent *Report of the AVMA Panel on Euthanasia*[1]. The use of inhalant agents for euthanasia must observe the conditions and precautions in the pertinent sections of that report, and to NIH guidelines in their latest revisions.

Species	**Age**	**Methods**	**Notes**
Mouse Rat Hamster Gerbil	0-14 days gestation	Euthanize dam	-
	15 days gestation to birth	• Barbiturates OR • Cervical dislocation OR • Decapitation OR • Overdose of injectable anesthesia	Euthanize dam with carbon dioxide. Fetuses are resistant to inhalant euthanasia at this stage and must be euthanized with one of the methods listed on the left. If age of gestation is unknown, use procedure for 15 days to birth.
	< 6 days old	• Ice water/hypothermia anesthesia, THEN secondary method	Place pup in finger of disposable glove, place in ice water until unresponsive; immediately employ secondary method.
	0-10 days old	• Anesthetic overdose (Ketamine/Xylazine, Barbiturates) OR • Isoflurane/sevoflurane • THEN, cervical dislocation/decapitation under anesthesia	At this state, neonates are resistant to carbon dioxide euthanasia.
	Adult (>10 days old)	• Barbiturates OR • Inhalant anesthesia (CO2, isoflurane sevoflurane) • THEN cervical dislocation/decapitation	Cervical dislocation is appropriate for rats and mice less than 200g. Decapitation is acceptable when justified by experimental conditions.
Species	**Age**	**Methods**	**Notes**
Guinea pig	3-34 days gestation	Euthanize dam	-
	35 days gestation to birth	• Anesthetic overdose (Ketamine/ Xylazine, Barbiturates) • THEN decapitation under anesthesia	At this stage, neonates are resistant to inhalant euthanasia.
	Birth → adult	• Anesthetic overdose (Ketamine/ Xylazine, Barbiturates) OR Carbon dioxide	Treat pups as adults.

Rabbits	Adult	• Anesthetic overdose (Barbituates) OR • Pre-sedate and inhalant anesthesia (including Carbon dioxide) THEN • Cervical dislocation or penetrating or non-penetrating captive bolt	For carbon dioxide, the displacement rate for rabbits is 50% to 60% of the chamber or cage volume/min. As fossorial animals, rabbits appear to have a higher tolerance for elevated CO_2 levels.
Zebrafish	≤ 7 days post fertilization (dpf)	• Rapid Chilling to 2° to 4°C (ice slurry) for ≥ 20 mins. THEN • Immersion in diluted sodium or calcium hypochlorite (1% final concentration) for ≥ 5 mins THEN • Freeze (disposal)	Nervous system at this stage regarded insensate to pain. Embryos at this stage may recover from ice slurry if exposure < 12 hrs.
	8 to 14 days post fertilization	• Rapid Chilling to 2° to 4°C (ice slurry) for ≥ 20 mins. THEN • Immediate freezing (disposal) OR • Decapitate and pith (for tissue harvest)	14 dpf may recover from ice slurry < 60 mins exposure.
	Adult (≥15 dpf)	• PREFERRED: Rapid Chilling to 2° to 4°C (ice slurry) for 10 mins (100% lethal). • ALTERNATIVE: Immersion in fresh, buffered (pH 7.0-7.5) Tricaine methanesulfate (tricaine mesylate, Tricaine-S, TMS, MS 222) solution (500 mg/L) for 30 minutes (attended). THEN • Immediate freezing (disposal) OR • Decapitate and pith (for tissue harvest)	Buffer the MS 222 with sodium bicarbonate (~2:1 weight ratio with MS 222). Check pH. Light sensitive. Does not store well. Make fresh. Concentrated stock solution (MS 222 10g/L) may store up to one month refrigerated. Prepare solutions under a fume hood and wear nitrile gloves, mask, and eye protection. Always use gloves. There is abrupt physiological maturation and increased susceptibility to Rapid Chilling between 14 dpf and 16 dpf.
Frog	larvae, and tadpoles	• Immersion in fresh, buffered (pH 7.0-7.5) Tricaine methanesulfate (tricaine mesylate, Tricaine-S, TMS, MS 222) solution (6g/L) for 15 minutes	Note: Rapid Chilling ineffective.
	Adult	• Intracoelomic co-injection of sodium pentobarbital (1100 mg/kg) plus phenytoin (141 mg/kg) solution, or 1100 mg/kg sodium pentobarbital solution (Fatal Plus® is 390 mg/ml sodium pentobarbital) and wait 3 hrs. OR • Immersion in fresh, buffered (pH 7.0-7.5) Tricaine methanesulfate (tricaine mesylate, Tricaine-S, TMS, MS 222) solution (high dose: 5 - 10g/L) for at least 60 minutes (attended). THEN • Decapitation *and* pithing or double-pithing with training and written IVET permission	With MS 222, anesthesia usually obtained within ~4-6 mins. Buffer the MS 222 with sodium bicarbonate (~2:1 weight ratio with MS 222). Check pH. Light sensitive. Make fresh for frogs – do not use stored concentrated stock solution. Prepare solutions under a fume hood and wear nitrile gloves, mask, and eye protection. Always use gloves.
* Other	-	-	Consult the Institutional Veterinarian

** USDA-covered species or species not listed*

As a means of euthanasia, administration of inhalant overdose results in deep depression of all life signs prior to death. It is possible that animals could revive from this state, which can be mistaken for death during a cursory examination. To prevent such an occurrence, the TTUHSC Animal Care and Use Committee has instituted the following policy.

Secondary Method

Administration of an inhalant overdose **must** be followed by one of the following secondary procedures:

- Cervical dislocation
- Decapitation
- Exsanguination
- Bilateral thoracotomy

Guidelines for Euthanasia of Rodents Using Carbon Dioxide

CO_2 inhalation is the most common method of euthanasia used for **mice, rats, guinea pigs, gerbils, and hamsters** and must be used as follows:

- The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber. All animals in the chamber must be able to make normal postural adjustments.
- Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide, as it allows the inflow of gas to the induction chamber to be controlled without pre-charging the chamber. Place the animal(s) in the chamber and introduce 100% carbon dioxide at a displacement rate of 30% to 70% of the chamber or cage volume/min (for rabbits, use 50% to 60% of chamber or cage volume/min). CO_2 flow should be maintained for at least 1 minute after respiratory arrest.
- Animals should be left in the container until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO_2 concentrations and exposure times or by other means.
- The use of dry ice for CO_2 euthanasia is NOT permitted.

Exceptions to these guidelines will be considered by the IACUC on a case-by-case basis.

References

1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
2. NIH Office of Animal Care and Use. Guidelines for Euthanasia of Rodent Fetuses and Neonates Rev 06/22/16
3. NIH Office of Animal Care and Use. Guidelines for the Euthanasia of Rodents Using Carbon Dioxide Rev 01/25/17

Policy 22: Use of Fertilized and Embryonated Avian Eggs

Any investigator intending to use fertilized and embryonated avian eggs/embryos (i.e. chicken eggs and other avian species where eggs are commercially available) before Day 15 of incubation need not have IACUC approval provided that the following criteria are met:

1. The PI must submit a Letter of Intent (LOI) to the IACUC, briefly stating what procedures will be performed on the embryonated eggs.

2. The Letter must state that the embryonated eggs will be used before Day 15.

3. The Letter must state a detailed plan for veterinary staff intervention in the event that any egg inadvertently hatches.

4. The Letter must state that veterinary staff assistance will be sought for humane euthanasia of any embryo that reaches Day 15 of development or beyond.

5. The Letter must state that the IACUC Chair will be notified when eggs are discovered that have reached Day 15 of development and/or have inadvertently hatched, in order to determine if corrective action is required.

Research, testing, teaching or training activities that require the use of embryonated avian eggs from Day 15 of development and beyond require an approved IACUC protocol.

Note: The use of embryonated eggs of avian species that are field-gathered (i.e., field studies) must be reviewed and approved by the IACUC.

Policy 23: Frog Oocyte Harvest

1. Background

Amphibian oocytes are used for studies of molecular biology, embryology and biochemistry. Stage I-VI oocytes are often obtained by surgical laparotomy. Multiple surgeries on a single animal may be justified considering the simplicity of the procedure, the lack of complications when performed by competent personnel, the effectiveness of anesthetic regimens, and reduction in the number of animals needed compared to the number that would be required if only one surgery were permitted.

2. Policy

Five recovery surgeries/animal (with a final 6th terminal surgery) will be permitted.[4] Waivers may be granted on an exceptional basis by the IACUC for compelling scientific reasons.

Adequate recovery time shall be allowed between laparotomies. Investigators shall alternate oocyte collection between right and left ovaries and rotate frogs so that the interval between surgeries is maximized. Recovery times shall not be less than one month under any circumstances unless approved by the IACUC.

3. Training

Surgeries must be performed by persons with appropriate training and must use appropriate anesthetic agents, such as tricaine methane-sulfonate (MS-222).

Professional and technical personnel and students who perform anesthesia, surgery, and euthanasia must be trained to accomplish these tasks in a humane and scientifically acceptable manner. The principal investigator must assure the IACUC that the project personnel have demonstrated competency or will be adequately trained to perform anesthesia, surgery, euthanasia and necessary monitoring to the satisfaction of the Attending Veterinarian or a qualified individual designated by the Attending Veterinarian. The LARC veterinary staff is available to provide assistance with, or training in, aseptic and surgical techniques, the proper administration of anesthesia, and euthanasia.

4. Xenopus Oocyte Harvest Recommended Protocol

A. Pre-operative considerations: Frogs are to be fasted for 6-12 hours to prevent emesis during anesthesia. Animals should be individually identifiable (i.e., RFID microchip implanted at the first surgery).

B. Anesthesia and supportive care during surgery: MS-222 can be safely used on Xenopus sp. at a dosage range of between 0.5 and 3 g/L. MS-222 solutions should be buffered to pH 7.0. Dosage selection is dependent upon the weight/size of the frog and the duration of anesthesia required. Once a surgical plane of anesthesia has been reached, (noted by a lack of response to deep pain, i.e. toe pinch) animals are placed in dorsal recumbency on the non-absorbent "blue" side of a clean/unused diaper pad. Optionally, investigators may lay the frog in an ice-filled container covered with plastic wrap (to prevent skin freezing) to induce hypothermia for additional analgesic effects. The frog can be frequently exposed to water containing dissolved MS-222 to maintain current level of anesthesia for long procedures (>30min.) or moistened less frequently for brief procedures (<30min). Frog skin must remain moistened throughout the procedure to prevent desiccation and precipitate complications. Take care not to introduce anesthetic water into the incision as this will prolong recovery from anesthesia.

C. Instrument Sterilization: Surgical instruments must be washed or soaked post-usage to remove all debris. They can be subsequently wrapped or packed appropriately before being sterilized with high heat and pressure or high heat alone (steam autoclave) or adequate exposure to gas (ethylene oxide). Steam is the preferred method (autoclaving). Instruments are to be stored in a dry place in which the integrity of the wrapping or packing material will be maintained for one year (sterilized packages must be clearly labeled with the one-year expiration date). Instruments sterilized in sealed plastic pouches are good for 1 year. Instruments wrapped and sterilized in cloth are good for 30

days. This extends to 6 months if heat sealed in plastic bags, 2 months if tape sealed in plastic bags. Instruments wrapped and sterilized in polypropylene cloth are good for 6 months.

Sterile materials are no longer considered sterile if the external packaging becomes wet or torn. If multiple surgeries are to be performed on different animals, then previously sterilized instruments may be "quick"-disinfected using a glass bead sterilizer (at least 15 sec). Instruments soaked in chemical disinfectants must be rinsed in sterile water or saline solution before their use on animals. No more than five successive surgeries may use instruments "quick"-disinfected as described above.

D. Surgical Skin Preparation: Skin asepsis in Xenopus sp. is not typically required for the most common surgical procedures performed on this species. However, it is recommended that debris should be removed only from the area immediately surrounding the surgical incision site (by a brief rinse with sterile saline, moistened small gauze pad or cotton swab). There is minimal potential for contamination of the surgical site and the development of subsequent post-operative infection if aseptic procedures are employed (use of gloves, a clean lab coat, and sterile instruments, and surgery occurs in a dedicated /previously disinfected surgical area on a clean surface).

If necessary, the recommended product for skin asepsis is 0.75% chlorhexidine or 2mg/L of benzalkonium chloride with a contact time of 10 minutes after removal of excess skin secretion from the surgical area. The surgical site shall be rinsed with sterile saline before the surgical incision is made. Products that contain soaps or detergents must be avoided and iodine-based products must be diluted significantly if they are to be used; e.g., 0.5% betadine solution.

Care must be taken not to scrub the skin too vigorously as this may remove the protective mucous layer, leading to post-operative complications.

E. Surgical Procedure: A small, paramedian, coelomic incision (0.5-2cm) through the skin and muscular layers is made on either the right of left side of the coelom. A portion of the corresponding ovary is exteriorized and removed. Remaining ovarian tissue is re-inserted into the coelomic cavity and checked for excessive hemorrhaging. The surgical wound is closed in two layers. The muscle layer is closed with absorbable suture in a simple interrupted pattern. The skin is closed with monofilament nylon non-absorbable suture in a simple interrupted pattern. The surgical site is monitored daily for 5 days and then every 2-3 days until skin sutures are removed at 10 days.

F. Post-surgical Recovery and Monitoring: It is the general consensus that post-surgical analgesia following oocyte harvesting is not necessary. It is felt that Tricaine (MS-222) may have analgesic properties. After surgery, the animal is allowed to recover for approximately 30-60 min in a container with a level of water not to cover the nostrils of the frog (desiccation of the skin on the dorsum of the frog can be prevented by placing moistened gauze on any exposed surfaces). Once the frog is active and mobile the water level can be raised to a more normal level and the gauze removed. Dechlorinated water is to be used during recovery from anesthesia and the container must be covered to prevent escape attempts. If dechlorinated water is not available, tap water containing 50g/L of non-iodized salt may be used (frogs must not remain in this type of water for longer than 12 hours). Frogs must be monitored daily for at least 5 days after surgery for evidence of excessive inflammation of the incision site, suture dehiscence, or abnormalities indicative of illness (anorexia, listlessness, lethargy, bloating, or "red-leg"). If evidence of wound infection or illness is noted then LARC vet services are to be contacted for evaluation and treatment or the animal shall be euthanized.

Single housing or small-group housing for several days after surgery shall be provided as part of the post-surgical care of laparotomized animals. Frogs shall be monitored daily during the post-operative period (at least 5 days) for the appearance of a normal appetite as well as for any complications such as dehiscence or infection. The appearance of such adverse effects provides justification for immediate euthanasia.

G. Euthanasia: Consist with IACUC Policy 21, Euthanasia, may be achieved either by intracoelomic co-injection of sodium pentobarbital (1100 mg/kg) plus phenytoin (141 mg/kg) solution, or 1100 mg/kg sodium pentobarbital solution (Fatal Plus® is 390 mg/ml sodium pentobarbital) and wait 3 hrs. OR immersion in fresh, buffered (pH 7.0-7.5) Tricaine methanesulfate (tricaine mesylate, Tricaine-S, TMS, MS 222) solution (high dose: 5 - 10g/L) for

at least 60 minutes (attended). THEN followed by Decapitation and Pithing or Double-Pithing with training and written IVET permission.

If using MS 222, buffer the MS 222 with sodium bicarbonate (~2:1 weight ratio with MS 222). Check pH. Light sensitive. **Make fresh** – do not use stored concentrated stock solution. Prepare solutions under a fume hood and wear nitrile gloves, mask, and eye protection. Always use gloves.

Please contact vet services if assistance with the pithing technique is required.

H. Surgical Records: A "Surgical Record" shall be completed immediately after the surgical procedure. Records may be in composite (group) format and can be included as part of research data collected, but shall be available for review.

Records shall identify the individual animal identification, type of surgical procedure performed, the date of the procedure, the person who performed the procedure (or initials), information on drug administration and peri-operative monitoring, and shall be maintained in the laboratory. This information shall be available for review by regulatory bodies, including the IACUC.

5. Exceptions

Any and all deviations from this policy shall be presented to and approved by the IACUC.

References

1. Guidelines for Egg and Oocyte Harvesting in Xenopus Laevis. Revised 10/26/2016. ARAC Guidelines, https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/oocyte_harvest.pdf
2. Suckow MA, et al. 1999. Evaluation of hypothermia-induced analgesia and influence of opioid antagonists in Leopard frogs (Rana pipiens). Pharmacol Biochem Behav 63: 39–43.
3. Wright, K.M., B.R. Whitaker. 2001. Amphibian Medicine and Captive Husbandry. Krieger Publishing Co.
4. Silverman, J., Suckow, M.A. & Murthy, S. (2014). *The IACUC Handbook*, 3rd ed. 18:13, 427-428.

Policy 24: Use of Zebrafish for Research and Teaching

1. Background

This document is intended to assist researchers/instructors working with zebrafish in determining when Institutional Animal Care and Use Committee (IACUC) review is required. These guidelines were adopted from several sources, including the *Guidelines for Use of Zebrafish in the NIH Intramural Research Program*.

2. Definitions and General Information[3]

Embryos - Fish at 0-3 days post-fertilization (dpf).

Hatchlings - The NIH Office of Laboratory Animal Welfare (OLAW) considers fish species to be "live vertebrate animals" at "hatching". Although this is an imprecise stage in zebrafish, OLAW considers zebrafish hatching to occur at 72 hours or 3 (dpf).[4]

Early Larval Stages - The larval stage begins after hatching and proceeds until Day 30. There is no evidence to suggest the presence of higher order cognition in zebrafish during the first week of development[1]. While the capacity for suffering is the primary criterion for establishing a threshold for 8 dpf for euthanasia in zebrafish, the criterion of independent feeding (and not feeding from the yolk sac, which continues through 7 dpf) also supports this age.

Older fish (larvae, juveniles and adults) (≥ 8 dpf) - From Days 30-90 the fish are called "juveniles," and from Day 90 onward, they are considered as adult fish[3].

Many studies have demonstrated that older zebrafish show evidence of higher order cognition, including learning to avoid aversive stimuli. Still, the ability of older fish to experience suffering remains controversial in the scientific literature. However, there is sufficient evidence to take a cautious approach in older zebrafish by instituting guidelines that ensure rapid euthanasia.

For the purposes of animal studies that need IACUC approval, the most important developmental stages are the embryo, 4–7 dpf, and 8 dpf and beyond.

3 Protocol Procedures

Zebrafish embryos are not considered live vertebrate animals and do not need to be included in your IACUC protocol. However, a description of their use may be necessary as part of a complete description of experimental protocols involving adult breeding zebrafish. In addition, guidelines on euthanasia must be followed for both embryos and adults.

Zebrafish early larvae (> 3 dpf) are considered live vertebrate animals and must be included in the IACUC protocol, with their numbers justified. Because early stage animals (3–7dpf) do not feel pain or distress, the researcher/instructor may check "No" for the relevant pain and distress section of the protocol application. For the IACUC approval process, applications using these early stage animals may designate the protocol as a "Category C". The pain and distress categorization of the ≥8 dpf fish should be determined according to the specific procedures performed as described in the protocol.

4. Euthanasia Guidelines[2]

A. For embryos ≤ 3dpf (i.e., before hatching), development should be terminated using bleach as follows:
Addition of bleach solution (sodium hypochlorite 6.15%) to the culture system water at 1 part bleach to 5 parts water. The embryos should remain in this solution for at least five minutes prior to disposal to ensure death. As detailed above in the scientific background section, pain perception has not developed at this early stage so this is not considered a painful procedure.

B. For zebrafish 4–7 dpf the following methods are acceptable for euthanasia:
Immobilization by submersion in ice water (5 parts ice/1 part water, 0–4º C) for at least 20 minutes to ensure death by hypoxia. Euthanasia may also be accomplished using bleach as described above in item 4.A.

C. For zebrafish ≥ 8 dpf the following methods are acceptable for euthanasia:
Immobilization by submersion in ice water (5 parts ice/1 part water, 0–4º C) for at least 10 minutes following cessation of opercular (i.e., gill) movement. In any fish where it is difficult to visualize opercular movement, fish should be left in the ice water for at least 20 minutes after cessation of all movement to ensure death by hypoxia.

Overdose of tricaine methane sulfonate (also known as MS222 [CAS no. 886-86-2], an FDA-approved fish anesthetic, 200–300 mg/l) by prolonged immersion. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement.

Anesthesia with MS222 at 168 mg/l, followed by rapid freezing in liquid nitrogen.

Decapitation with a sharp blade by a trained individual when its use is required by the experimental design and approved by the IACUC.

D. Zebrafish carcasses should be disposed of according to normal IACUC procedures.

References

1. *Guidelines for use of zebrafish in the NIH intramural research program*. Bethesda, MD: NIH, 2009. Available at: https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/zebrafish.pdf. Rev 06/22/2016
2. National Institutes of Health (2009) Final Report to OLAW on Euthanasia of Zebrafish.
3. Westerfield, M. 2000. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish Danio (Brachydanio) rerio*. 5th ed, Univ. of Oregon Press, Eugene. http://zfin.org/zf_info/zfbook/zfbk.html
4. *Zebrafish* 2016; 13(6):1-2, OLAW Comments. https://grants.nih.gov/grants/olaw/references/zeb_2016_1344.pdf