GUIDELINES
FOR THE USE OF ANIMALS
IN RESEARCH AND TEACHING
Table of Contents

I) OVERVIEW ........................................................................................................... 4
   A) ANIMALS COVERED UNDER THE SAINT MARY’S COLLEGE OF CALIFORNIA THE IACUC PROTOCOL ............................................. 4
   B) POLICIES ........................................................................................................ 4
   C) COMPLIANCE ............................................................................................... 5
   D) REFERENCES: .............................................................................................. 6

II) INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) .................................................. 7
   A) RESPONSIBILITIES .................................................................................... 7
   B) PRINCIPAL INVESTIGATOR (PI) : RESPONSIBILITIES ............................... 8
   C) SAINT MARY’S COLLEGE ADMINISTRATION: RESPONSIBILITIES .......................... 8

III) IACUC REVIEW PROCESS ............................................................................. 9
   A) APPROVAL PERIOD .................................................................................. 9
   B) TYPES OF REVIEW .................................................................................. 9
   C) TYPES OF MODIFICATIONS ..................................................................... 10
   D) APPROVAL PROCESS .............................................................................. 10
   E) TYPES OF REVIEW OUTCOMES .............................................................. 11

IV) NEW APPLICATION FOR IACUC APPROVAL ............................................ 12
   A) SCIENTIFIC MERIT .................................................................................. 12
   B) ANIMAL CATEGORIES.............................................................................. 12

V) IACUC MANDATED TRAINING FOR RESEARCHERS USING ANIMALS IN THEIR RESEARCH 13

VI) IACUC POLICY ON RESEARCHER NON-COMPLIANCE ................................. 14
   A) PURPOSE .................................................................................................. 14
   B) AUTHORITY .............................................................................................. 14
   C) POLICY .................................................................................................... 15
      i) Determination of Non-Compliance ......................................................... 15
      ii) Classification of Non-Compliance ....................................................... 15
      iv) Consequences and Resolution of Significant Non-Compliance Incidents ............................................................. 16
      v) Federal Reporting of Non-Compliance ............................................... 16
   D) REFERENCES .......................................................................................... 18

VII) IACUC GUIDELINES .................................................................................. 19
   A) ZEBRAFISH INCLUSION IN IACUC PROTOCOLS ........................................ 19
   B) ANESTHESIA GUIDELINES- RODENTS .................................................. 20
   C) SURGERY GUIDELINES – RODENTS ....................................................... 22
   D) MAINTAINING ANIMAL STUDY AREAS (RODENTS) ................................. 25
   E) EUTHANASIA GUIDELINES FOR RODENTS ........................................... 27
   F) GUIDELINES FOR EUTHANASIA OF ZEBRAFISH ................................. 31
   G) COHOUSING ........................................................................................... 32
   H) RAT CAGE DENSITY ................................................................................ 33
   I) MOUSE CAGE DENSITY .......................................................................... 34

VIII) IACUC STANDARD PROCEDURES .............................................................. 35
   A) OVARIECTOMY IN RATS AND MICE ...................................................... 35
   B) VASCULAR PERFUSION WITH FIXATIVES FOR TISSUE COLLECTION ............................ 37
   C) BAR HOLDING FOR GRIP STRENGTH ..................................................... 38
   D) FORCED EXERCISE/WALKING WHEEL SYSTEM .................................. 39
   E) CROSSING ZEBRAFISH .......................................................................... 40
   F) OOCYTE COLLECTION FROM XENOPUS LAEVIS FROGS ......................... 41
Research and Instruction using Animal Subjects

I) OVERVIEW

Animal use in research and teaching is essential to provide students with the expertise they need to continue into careers and further education in agriculture, veterinary medicine and other biological sciences. The use of animals in research has led to many significant discoveries in the field of biology and medicine. Therefore, it is important to expose students to animal research while ensuring the humane treatment and use of animals for research and teaching. Each faculty member, student and staff member involved in the care of the animals is responsible for the welfare and humane treatment of the animals during the research or instructional use at Saint Mary’s College of California. Therefore, Saint Mary’s College of California has established policies and guidelines for the humane treatment and use of animals in research and teaching. These guidelines are in compliance with the applicable federal and state regulations.

The animal care and use guidelines are adapted from the Animal Care and Use Guidelines at UCSF, with their permission.

A) Animals covered under the Saint Mary’s College of California the IACUC protocol

Rodents (Rats, mice, Hamsters, Guinea pigs)
Chick (after birth)
Amphibians (Frogs (Xenopus, Rana, Buffo), toads, Salamanders, Mudpuppies), Zebrafish

B) Policies

1) The use of animals in teaching and research should be limited to experiments that demonstrate principles, obtain new research information that will ultimately benefit the society. The use of animals should be supplemented with other methods whenever feasible – such as models, in vitro systems. The investigator or instructor should explore possible methods to reduce and replace the animal model, if possible.

2) All research and teaching activities using vertebrate animals will be reviewed by the Institutional Animal Care and Use Committee (IACUC). The IACUC committee will apply the guidelines in the Animal Welfare Act and the Health Research Extension Act of 1985 (and the revisions) to review and approve or withhold approval of the proposed use of animals.

3) The procurement, care and use of animals will be in accordance to the guidelines in the Animal Welfare Act and the Health Research Extension Act of 1985 (and the revisions).
4) The experimental procedures using animals will be planned and conducted in a manner as to minimize pain and distress to the animals. These procedures will be performed by trained personnel (typically a faculty member). The students participating in the procedure must be adequately trained in the procedure and supervised. If any experimental or demonstrative procedure, or its consequences, have the potential to induce significant and/or lasting pain, distress or suffering, appropriate methods of tranquilization, anesthesia and analgesia must be used. Any painful or distressful procedure, regardless of whether it can or cannot be obviated, must be reviewed and approved in advance by the Institutional Animal Care and Use Committee.

5) Euthanasia on the animals should be performed in a manner consistent with the latest recommendations of the American Veterinary Medical Association Panel on Euthanasia. All the proposed methods must be approved in advance by the Institutional Animal Care and Use Committee (IACUC).

6) Any faculty member, staff member or student who believes or knows any violation to this policy can submit a written complaint to the Chairperson of the Institutional Animal Care and Use Committee for review of the particular procedure. The Committee will review the pertinent facts and report their findings to the appropriate administrative official.

C) Compliance

In order to ensure compliance, lab technicians will be trained to see and care for the animals housed in the facility. The laboratory protocols will be audited randomly by compliance personnel. Also, the IACUC with inspect the animal facilities at least once a year. Once a protocol is approved, all personnel with less than one year working with the species are required to participate in a mandatory training that must be completed before the work begins.

If a deviation from a research protocol or discomfort in an animal is noted by the IACUC committee members, they are responsible for ensuring that any issues regarding animal well-being is addressed. It is also documented in the animal's medical record. The incident is investigated by the IACUC. The director of the IACUC then submits a formal letter to the director of the Office of Laboratory Animal Welfare, signed by the Saint Mary’s College of California official, which is part of the National Institutes of Health (NIH). The process is transparent, as reflected in the 79 U.S. Office of Laboratory Animal Welfare (OLAW) reports, which were made over a period of five years.
D) References:

1) USDA Animal Care Policy

2) Public Health Service Policy on Humane Care and Use of Laboratory Animals
   http://grants.nih.gov/grants/olaw/references/phspol.htm

3) Guide for the Care and Use of Laboratory Animals
   http://www.nap.edu/catalog.php?record_id=12910

4) Health Research Extension Act of 1985

5) AVMA Euthanasia Guidelines

6) IACUC.org (http://www.iacuc.org)
II) Institutional Animal Care and Use Committee (IACUC)

To ensure the compliance of the guidelines, Saint Mary’s College of CA has set up a committee – The institutional Animal Care and Use committee (IACUC) to oversee the compliance with the federal and state guidelines.

The committee at Saint Mary’s College shall consist of not less than 4 members and shall include at least:
- a Chairperson;
- one Doctor of Veterinary Medicine
- one practicing scientist experienced in research involving animals;
- one member whose primary concerns are in a nonscientific area; and
- one individual who is not affiliated with the institution in any way other than as a member of the IACUC.

This Committee will be appointed by the Dean of the School of Science.

A) Responsibilities

1) Review all the applications for proposed use of live vertebrates for research and teaching.
2) Notify investigators and the Dean of the School of Science in writing its decision to approve or withhold approval of proposed animal use procedures.
3) Inspect the animal care facilities once in six months.
4) Ensure that the personnel conducting animal procedures are trained in animal use and in the procedures.
5) Review the institutional program for humane care and use of animals in research and teaching every 6 months.
6) Review concerns involving care and use of animals.
7) Suspend activities that are found to be not in compliance with the federal or state guidelines.
8) Provide information and recommendations to the Dean of the School of Science, Vice Provost for Undergraduate Affairs and the Provost regarding different aspect of animal program, facilities or training of personnel.
9) Submit reports on animal utilization activity to federal oversight regulatory agencies including the USDA and the National Institute of Health’s Office of Laboratory Animal Welfare (OLAW).
B) Principal Investigator (PI) : Responsibilities

Before initiating, modifying, or renewing any research project that uses animal subjects, Principal Investigators must submit an application to the Committee on Animals Research (IACUC) for review and approval. Principal investigators are also responsible for renewing their IACUC approvals on a yearly basis.

Principal investigators are responsible for assuring that everyone under their supervision involved in the care and use of laboratory animals is properly trained, is thoroughly knowledgeable about the details of the approved protocol, understands his or her obligation to comply with all applicable regulations and federal and institutional policies.

Anyone who witnesses or has reliable information about non-compliance with an IACUC-approved protocol or an incident of mistreatment of animals should notify the Chair of the Institutional Animal Care & Use Committee.

C) Saint Mary’s College Administration: Responsibilities

The Dean of the School of Science must assure the federal government on an annual basis that Saint Mary’s College of CA is in compliance with all provisions of the Animal Welfare Act, the NIH Guide for the Care and Use of Laboratory Animals, and the PHS Policy on the Humane Care and Use of Laboratory Animals.

The college provides the resources to support the activities of the IACUC, maintain necessary records, and report as required on the status of the animal care and use program and the membership of the Institutional Animal Care & Use Committee.

The college provides legal protection for members of the Institutional Animal Care & Use Committee and to principal investigators granted approval to conduct such research who have met their obligations in good faith.

The Institutional Official (IO) must report to the NIH’s Office of Laboratory Animal Welfare (OLAW) and the Animal Care section of the United States Department of Agriculture on the circumstances and actions taken in respect to:

• any serious or continuing protocol violation;
• any serious deviations from the provisions in the Guide for the Care and Use of Laboratory Animals; or
• suspension of any activity by the Institutional Animal Care & Use Committee.

Under the provisions of Freedom of Information Act and the California Public Records Act, the college is required, upon request, to release to the public details of any specific, funded research project.
III) IACUC Review Process

A) Approval Period

The proposals for using animals for a research study will be approved for a maximum period of 5 years. The IACUC will send courtesy reminders to PIs approximately 75 and 45 days before the expiration date of an approved study to allow sufficient time for renewal processing. Note: If renewal applications are not renewed and approved by the expiration date and there are animals being used for the study, the Principal Investigator and his/her staff will not have access to the animals until the application has been approved.

B) Types of Review

SMC-IACUC has adopted formalized procedures, known as “Designated Reviewer”, approved by the USDA and PHS. This process allows for review of proposed activities (protocols) by less than a full Committee.

Full Committee Review: Initial applications, and major changes (see below) in approved protocols are reviewed by the full committee and require completion of the Full Committee Application.

Designated Subcommittee Review: Applications for renewal after initial review and approval, are reviewed by the IACUC chair who can require a sub-committee to review the changes if necessary. For renewal application, the PI will need to complete the renewal form if the changes to the proposal are minor such as personnel changes, updates to animal numbers and minor edits to proposal. If a major modification to the protocol is involved, the PI needs to submit a complete application (see below under Modification review).

Urgent Reviews: Under certain rare circumstances the IACUC Chair may designate a reviewer for urgent reviews. The investigators must provide compelling reasons for these types of reviews.

Tissue Collection: The IACUC Chair may designate a reviewer for protocols proposing tissue collection only.

Modification Review: If the PI wishes to make modifications to the approved protocol during its approval period or during renewal period, the PI needs to submit a complete application form for a Full committee review. These changes must be approved before they are initiated. Approval for a modification does not change the existing expiration date if within an approval period.

Loss of Quorum: If a quorum is lost during a convened meeting of the Full Committee due to the early departure of one or more Committee members, the IACUC Chair may call for a vote by the Full Committee (before any members are excused) to review the remaining protocols on the agenda via the designated member review process.
C) Types of Modifications

**Administrative Modifications** such as addition or deletion of personnel, change in study title, change in location, are reviewed and approved by the chair of the IACUC.

**Other Modifications** such as changes in the original objectives, experimental procedures, animal numbers or species, experimental agents, therapeutic agents, or anesthetic/analgesic regimen are reviewed by Designated Member Review or the Full Committee depending on the extent of the modification as determined by the chair of the IACUC or a designated pre-reviewer.

*Modifications to the protocol may not be implemented until reviewed and approved by the IACUC.*

D) Approval Process

The IACUC approval process for a new application takes from four – six weeks and includes the following steps:

1. Once you submit your application, it will be pre-reviewed by the chair of the IACUC or a designated pre-reviewer to make sure that it is clear and complete and that all administrative requirements are met. You may receive an e-mail requesting revisions.
2. Once you revise your application according to the pre-review comments, you must resubmit it. If it is not accepted by the pre-reviewers for Full Committee review, you will be asked for further revisions. If it is accepted for Full Committee review, it will be sent to Committee members one week prior to the next scheduled meeting.
3. The Committee will review your application, and you will receive an approval letter, or a letter asking for further revisions to the application and your written response.
4. Once you have submitted your revised application, your response letter, and any additional required documents (e.g. P.I. certification page, evidence of scientific merit review) your response will be referred to the Committee Chair (unless the Full Committee wishes to review it again) for approval.

*To avoid problems with funding agencies or expiration of previous approvals, please be sure to allow enough time for all these procedures.*
E) Types of Review Outcomes

After IACUC review, one of the following determinations is made for each application:

**Full Approval** is granted when the Committee has no concerns about the application. The investigator is sent a letter with a IACUC approval number, valid for one year, and may begin the project.

**Revisions Requested** prior to approval is given when the members require a written response from the investigator. The members may ask the investigator to clarify a point, provide further information, or make revisions in the protocol. The investigator’s response is normally reviewed by the IACUC Chair. No approval is given until the questions and/or concerns of the Committee have been satisfactorily addressed and approved by the Chair.

**Returned for Additional Information** prior to committee re-review is requested when serious concerns are raised and the members agree that additional information and/or justification is needed before approval can be reconsidered. The investigator’s response must include a point-by-point letter addressing all concerns, as well as a revised application. The response is then reviewed by the Full Committee.

**Disapproval** is given when the Committee completely refuses to approve a protocol.

On rare occasions, the Committee may encounter major difficulty in making an assessment and an outside reviewer may be asked to consider the protocol.
IV) New Application for IACUC Approval

A) Scientific Merit

The IACUC will not review proposals for Scientific Merit but requires that each PI submit a statement that addresses the following areas pertaining to Scientific Merit of the proposal. These areas include (adapted from the NIH peer review process):

- **Significance**: does the project address an important problem in the field?
- **Investigator**: are the PI and other researchers capable of carrying out the project?
- **Innovation**: do the experiments challenge current scientific paradigms?
- **Approach**: are the overall strategy, methodologies, and analyses appropriate to accomplish the specific aims of the project?
- **Environment**: is the scientific environment adequate for successful completion of the work?

B) Animal categories

Per federal regulations, experimental procedures are categorized according to their potential for causing pain or distress to animal subjects. You must designate categories for all study animals.

<table>
<thead>
<tr>
<th>USDA Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Animals upon which teaching, research, experiments, or test were conducted involving no pain, distress, or use of pain-relieving drugs.</td>
</tr>
<tr>
<td>D</td>
<td>Animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.</td>
</tr>
<tr>
<td>E</td>
<td>Animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to the USDA Annual Report).</td>
</tr>
</tbody>
</table>

*An animal is counted:*

* only once per year, even if it was used in more than one Protocol
* in the most painful/distressful Category, if used in more than one category
V) IACUC MANDATED TRAINING FOR RESEARCHERS USING ANIMALS IN THEIR RESEARCH

IACUC at Saint Mary’s College of CA requires an in-person/online training session for any researcher or personnel involved in animal care and use.

Training topics include:

1. Policy/Guidelines Review:
   - Policy: Protocol Requirements for Animal Housing and Study Areas
   - Guidelines for the Laboratory Housing of Research Animals
   - Guidelines: Maintaining Animal Study Areas

2. Documentation requirements:
   - Daily tasks are documented every day animals are present.
   - In the event of intermittent animal housing, researchers are instructed on how to complete the form so that it indicates there are no animals housed.
   - Information is provided on what is expected of laboratory care personnel if temperature/humidity/light cycle values fall outside of the specifications within The Guide.
   - Daily check sheet retention for a reasonable time to facilitate IACUC review

3. Feed, water, drugs, and materials: labeling, storage and inventory management practices, including establishing a program for ensuring items are within date.

4. Caging
   - Enrichment, social housing, identification, cage space requirements

5. Cleaning and Sanitization
   - A Cleaning/sanitization SOP is in place or will be developed by the lab after reviewing any site-specific needs during this training session.
   - Cage change/water bottle change intervals

6. Health monitoring
   - Review general clinical signs of pain/distress/disease
   - Contacting emergency veterinary care when needed

7. Proficiency demonstration
   - The operation and resetting of a thermometer/hygrometer
   - Maintenance of the appropriate light cycle

8. The following required postings will be provided by IACUC:
   - emergency on-call veterinary care information
   - Sign for reporting Animal Care and Use Concerns
   - IACUC Policy on Expired Drugs and Materials Used in Animal Research
   - Ensure that a pest control program is in place
VI) IACUC POLICY ON RESEARCHER NON-COMPLIANCE

A) PURPOSE

The IACUC is required to ensure humane care and use of vertebrate animals used for research, instruction, and testing, and to adhere to the applicable federal and state regulations and institutional policies affecting the use of animals at Saint Mary's College of CA. As a result, the IACUC monitors the animal care and use program and its various components for compliance with all the appropriate regulations and policies.

The IACUC has developed this policy for evaluating issues of non-compliance with the IACUC protocols, policies and regulatory guidelines. Although uniform standards can serve as a guide, each individual case is unique and will be judged on its own merits.

B) AUTHORITY

The IACUC is charged with and is responsible for ensuring compliance with federal regulations under Campus Administrative Policy 100-17, Research and Instruction Using Animal Subjects. In order to fulfill these obligations, the IACUC must exercise authority granted by the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals and the Animal Welfare Act and Regulations (AWAR) to enforce policies and regulations.

Under the PHS Policy on Humane Care and Use of Laboratory Animals, the IACUC must promptly report (via the Institutional Official) to the National Institutes of Health (NIH) Office of Laboratory Animal Welfare (OLAW) the following:

- Any serious or continuing noncompliance with the PHS Policy;
- Any serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals (the Guide); and
- Any suspension of an activity by the IACUC.

The AWAR also requires that if the IACUC suspends an activity involving animals, the Institutional Official, in consultation with the IACUC, will review the reasons for suspension, take appropriate corrective action, and report that action with a full explanation to the United States Department of Agriculture (USDA) and any Federal agency funding that activity.
C) POLICY

i) Determination of Non-Compliance

To determine whether an issue should be classified as non-compliance, the IACUC will initiate an investigation involving the Principal Investigator (PI). If the activity in question is determined to be non-compliant, it will be categorized based on its severity (as described below), a Compliance Report will be presented to the IACUC, and necessary corrective actions will be taken.

Minor issues involving animals are not always initially deemed as non-compliance. If such issues are observed by the IACUC staff, then the investigator or his or her staff will be notified and given an opportunity to address it to avoid a finding of non-compliance.

Self-reporting is encouraged. Self-reporting gives the IACUC notice and opportunity to investigate and the PI a chance to remedy the issue before it becomes non-compliance or allows the PI to inform the IACUC of self-corrective measures taken to prevent recurrence. Monitoring and clarification of institutional policies and guidelines regarding the care and use of animals is routinely provided by the IACUC to assist the investigators and to maintain overall institutional compliance.

ii) Classification of Non-Compliance

The IACUC classifies non-compliance incidents into one of two categories: Significant and Minor. Both categories are defined below with examples of non-compliances, as well as an outline for corrective actions that has been delineated by the IACUC. The examples are provided as a general guideline to help PIs and research staff in understanding this process. As situations vary considerably, determinations are made on a case-by-case basis based on the totality of the circumstances, including self-reporting, voluntary corrective actions, and any other relevant considerations.

a) Significant Non-Compliance

Significant non-compliance indicates a serious breach of laws, regulations, or university policy, which compromises the function of the IACUC or puts researchers or animals at risk of undue harm.

Examples of Significant non-compliance include, but are not limited to:

- Acquiring animals for research or performing unapproved procedures without the IACUC approval;
- Performing a procedure in such a manner that animals endure pain or suffering that is not addressed by the approved protocol;
• Performing a procedure with improper technique or safeguards which puts either the staff or animals at risk;
• Failure to adhere to proper aseptic technique for survival surgery;
• Repeated or willful incidents of minor non-compliance.
• Failure to provide adequate anesthesia or analgesia according to protocol

iv) Consequences and Resolution of Significant Non-Compliance Incidents

If an occurrence of non-compliance directly results in a significant negative impact to animal welfare, the Attending Veterinarian or the IACUC Chair or their designate(s) have the authority to immediately stop all procedures necessary to protect the health and welfare of the animals. The PI will be contacted as soon as possible and the matter will be referred to the IACUC and Institutional Official for further investigation.

Within the category of significant non-compliance, incidents will vary in their degree of seriousness. An IACUC subcommittee may recommend a corrective action, and a majority vote by a quorum of IACUC members will decide on a corrective action and may vote on protocol suspension, revocation of research privileges, or other sanctions.

If the IACUC votes for protocol suspension, all procedures and ordering privileges encompassed by that protocol must cease during the period of suspension. If a protocol is suspended, the PI and his/her department chair will be contacted as soon as possible and a letter will be sent to the PI from the IACUC that requires a response regarding corrective action and future preventive measures. The PI also may be asked to meet with the IACUC, the IACUC Chair and/or the Institutional Official as a condition of reinstatement.

Following an investigation that includes a full discussion of the situation with the PI; the IACUC will meet to determine the corrective actions that will be required of the PI. A majority vote of the IACUC with a quorum present, in consultation with the Institutional Official, may lift a suspension only after it has been determined that the protocol's activities can be accomplished in full compliance with the relevant rules and regulations and that adequate measures have been taken to prevent recurrence of the non-compliant activity.

v) Federal Reporting of Non-Compliance

In the event of an incident of significant non-compliance, during the initial investigation the IACUC will determine the extent of the non-compliance. Incidents of significant non-compliance that are deemed serious or continuing or represent a serious deviation from the provisions of the Guide, including suspensions, will require reporting to the OLAW through the the Dean of the School of Science or their designee and may be reported to the federal funding agency supporting the activity.
b) Minor Non-Compliance

Minor non-compliance typically arises in instances where policy has been violated but the risk of harm to researchers or animals is minimal and the IACUC authority or function has not been compromised. Minor non-compliance can often be corrected at the institutional level.

Examples of minor non-compliance include, but are not limited to:

- Not informing the IACUC of the addition of personnel;
- Not maintaining surgical and post-operative care records per IACUC policy and/or protocol requirements;
- Failure to respond to a "Health Check" card and address the problem or failure to monitor the animals adequately following invasive procedures;
- Use of an unapproved procedure area resulting in failure of the IACUC to inspect this area as required by law or policy;
- Failure to observe approved timeframe for animals to be in off-site areas (i.e. laboratories);
- Personnel not attending training within the required time frame or not maintaining updated occupational health forms;
- Personnel accessing facilities without authorization;
- Inadequate controlled substance logs or controlled substance storage;
- Unapproved transfer of animals from one protocol to another;
- Improper or unapproved animal transportation;
- Not following safety procedures when working in BSL-2 or lower such as not wearing appropriate personal protective equipment;
- Housing animals in a lab without approval or over the time limit approved in the protocol;
- Inadequate housing conditions, e.g. overcrowded cages, weaning delays, or failure to separate aggressive animals as required;
- Minor protocol deviation, which does not significantly compromise animal welfare.
- Use of or failure to dispose of expired drugs;
- Use of expired medical materials without IACUC approval.

Consequences and Resolution of Minor of Non-Compliance Incidents
Resolution of minor issues may be achieved through communication between the LARC personnel, the IACUC staff, and the individual lab personnel without necessitating IACUC intervention.

If an issue is not resolved or is deemed more serious, it will be reported to the IACUC.
following notification process may be used to obtain compliance or escalate the non-compliance.

**First notification:** The PI will be required to provide a written response regarding how the incident occurred, how it was corrected and how it will be prevented in the future. This response will be reviewed by the IACUC, and the IACUC may also require additional steps including retraining of investigative staff member(s).

**Second notification:** Possible revocation of animal ordering or facility access privileges depending upon the circumstances and the response of the PI. The PI may be required to appear before the IACUC or a subcommittee of the IACUC.

**Third notification:** The Non-compliance may be reclassified as significant (see above). If reclassified, it may result in any potential consequences of significant non-compliance listed above. This may include sanctions up to and including a suspension of activities following a majority vote of the IACUC with a quorum present and/or revocation of a researchers privileges and any animal activity therein. The PI may also be required to meet with the IACUC Chair and/or the Institutional Official. A third notification may require reporting to the appropriate federal authority and any suspension of animal activity must be reported (see above).

**D) References**


4 Office for Laboratory Animal Welfare. Requirements for prompt reporting of problems to OLAW. OPRR Reports, January 12, 1994.


VII) IACUC GUIDELINES

A) ZEBRAFISH INCLUSION IN IACUC PROTOCOLS

Public Health Service policy requires that all live vertebrate animals be included in the IACUC approved protocol. 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/3 days post fertilization (dpf).

1. Zebrafish 0-3 days post fertilization (dpf) are not considered live vertebrate animals and do not need to be included in Section C of your IACUC protocol. Description of their use may nonetheless be necessary for a complete description of animal activities (Section G: Procedures) and as part of the justification for numbers of adult breeding zebrafish (Section C: Animals).

2. Zebrafish > 3 dpf (i.e. 72 hours or older) are considered live vertebrate animals and must be included with their numbers justified in the IACUC protocol.

SPECIAL CONSIDERATIONS FOR PAIN CATEGORIZATION OF ZEBRAFISH

1. Zebrafish from 3-8 dpf are all considered Category C animals, as these stages have not been shown to perceive pain or distress.

2. Zebrafish >8 dpf are categorized based on the specific procedures described in the protocol.
B) ANESTHESIA GUIDELINES - RODENTS

1. **Acclimation period and health observation:** Animals should be acclimated for at least two days before major survival surgery or general anesthesia for survival procedures. A pre-anesthetic health observation should be performed prior to the procedure. This involves reviewing the animal’s general appearance, activity, respiration, and body weight or body condition score.

2. **Fasting:** Rodents are generally not fasted before anesthesia. Water is not withheld.

3. **Provide heat:** Rodents can quickly become hypothermic under anesthesia and during recovery from anesthesia. Preferred heat sources during and after procedures include circulating warm water blankets, chemical heat packs, and insulating methods (e.g. Saran wrap). All require careful monitoring, as rodents can easily overheat. Always place an insulating layer such as a towel between the animal and the heat source. Depending upon the species and procedure, monitoring of body temperature may be indicated.

   Use of heat lamps is discouraged as they may cause animals to overheat. If a heat lamp must be used, place the lamp far enough away from the animals to prevent hyperthermia, and provide a large enough recovery space that the animal can move if it becomes too warm. Maintaining a thermometer at the level of the animal to monitor ambient surface temperature can aid in hyperthermia prevention.

4. **Administering the Anesthetic:** Rodents may be anesthetized with injectable or inhalant agents, or with a combination of the two. An adequate, even depth of anesthesia should be maintained throughout the procedure. For inhalants, this can be accomplished by adjusting the vaporizer as necessary. For injectables, supplemental doses of the agent can be administered as needed. Anesthetic doses must follow the approved IACUC protocol.

5. **Apply eye ointment:** For anesthesia/sedation lasting longer than five minutes, eye ointment is required to prevent corneal damage due to loss of blink reflex.

6. **Monitoring Anesthesia:** Before surgery is started, ensure that the animal is adequately anesthetized by testing the pedal withdrawal reflex (foot pad pinch on both hind feet). If the foot pad pinch causes a response, supply additional anesthesia and re-test before starting the procedure. Anesthetic depth must be rechecked regularly for the duration of the procedure. Monitor the animal’s responsiveness to painful stimuli, character of respiration, and color of the ears, tail, gums, or foot pads, and adjust anesthetic depth whenever needed.
7. **Stay in the room** with anesthetized animals while they are on the procedure table. Do not immediately return recovering animals to a cage containing un-anesthetized animals. Animals should be placed in a separate recovery cage with half the cage on a heat source, and observed every 10-15 minutes until ambulatory. When the animal is able to walk normally, it can be returned to standard housing.

8. **Post-procedure monitoring**: Monitor animals for signs of distress or discomfort during and after recovery. Record any complications, and contact the local veterinarian if problems recur. Administer analgesics per the approved IACUC protocol. Animals experiencing post-procedural complications that cannot be alleviated should be euthanized using approved guidelines.

9. **Fluid administration**: Administration of warmed (i.e. body temperature) fluids can help animals maintain blood pressure and speed up recovery from anesthesia or surgery. Fluids may be given IP, intravenously, or subcutaneously.

10. **Record keeping** is required for anesthesia/sedation procedures lasting longer than 15 minutes from the administration of the anesthetic/sedative agent(s), and for any surgical procedure. **Minimum required documentation includes**: procedure date, individual animal or cage ID, procedure performed and all drugs administered. Surgical or anesthetic problems must be documented. Refer to the protocol for other record keeping requirements. Group records may be utilized for documentation purposes when all animals in a cage undergo the same procedure.

**Resources**: In case of emergency, the IACUC veterinarian can be reached at XXX-XXX-XXXX.
C) SURGERY GUIDELINES – RODENTS

Scope:

These guidelines apply to all surgical procedures performed on rodents.

Section 1 - Survival Surgery in Rodents
Section 2 - Non-Survival (Terminal) Surgery in Rodents

Section 1 - SURVIVAL SURGERY
Any surgery conducted on animals that are expected to recover from anesthesia is considered survival surgery.

General:

Refer to the IACUC Anesthesia guidelines for more details about rodent anesthesia. Survival surgery on rodents should be performed using sterile instruments and sutures, clean or sterile surgical gloves, and aseptic procedures to reduce microbial contamination of exposed tissues to the lowest practical level.

PROCEDURES

Pre-Operative:

1. Surgery should be conducted in an uncluttered, disinfected area that promotes asepsis during surgery. Sanitize the counter/lab bench with bleach solution, chlorhexidine, or Clidox before surgery. The use of alcohol is discouraged due to long contact time required to take effect (15 minutes). Use sterile drapes, clean absorbable pads or towels, and replace these materials after each surgery session.

   All instruments and implants must be sterilized prior to use with steam autoclave, glass bead sterilizer or ethylene oxide gas sterilization. Label packaged instruments and implants with date of sterilization.

2. As soon as the animal is anesthetized, shave/remove fur from the surgical site (anatomic site). Perform this procedure in a location separate from where the surgery is to be conducted (i.e. a separate lab bench).

3. Administer any pre-operative analgesics per the IACUC-approved protocol.

4. Apply ophthalmic ointment (eye lube) to the animal’s eyes to prevent corneal damage.

5. Disinfect the surgical site (anatomic site) with dilute chlorhexidine or betadine scrub. Three alternating scrubs each of disinfectant and alcohol (or sterile saline) are recommended. Alcohol by itself is not an appropriate skin disinfectant for surgery.
6. Surgeons should don a clean lab coat or scrub top and a mask. A surgical bonnet is also recommended. Wash and dry hands and/or apply foam or gel disinfectant before aseptically donning surgical gloves. Place a sterile drape over the non-sterile part of the animal to prevent contamination of instruments and suture from exposed fur.

**Note on gloves:** Sterile gloves are required if you will be touching the animal or the tips of your sterile instruments with your hands. If you will only be touching the handles of your instruments, then clean, non-sterile gloves are acceptable.

**Operative:**
1. Provide the animal with a heat source for the duration of the procedure. Circulating warm water blankets and heating pads are preferred over heat lamps. Always have a towel or drape between the heat source and the animal.
2. Test the depth of anesthesia by performing a toe pinch on both rear feet. If toe pinch elicits a response, adjust your anesthesia accordingly, and re-test before making your incision.
3. Monitor the animal's vital signs, such as respiratory pattern, skin/mucous membrane color, and depth of anesthesia. If the animal responds to stimuli during surgery, stop the procedure until additional anesthetic is administered and toe pinch elicits no response.
4. Handle sterile instruments aseptically to minimize contamination. A bead sterilizer may be used mid-procedure if an instrument becomes contaminated. Make sure instruments are cool to the touch prior to using on the animal. Hot instruments are extremely damaging to tissue.
5. Instruments and gloves may be used for a series of similar surgeries provided they are still clean and are re-sterilized between animals. Bead sterilizers can be used to re-sterilize instruments. Gloves should be changed if soiled; if not soiled, spray with disinfectant. A new set of autoclaved instruments should be used for every cage. Keep sterile instruments on a sterile field when not in use.
6. Close incision using appropriate techniques and materials. If thorax or abdomen has been opened, a two-layer closure should be performed.

**Post-Operative:**
1. Recover the animal according to the IACUC anesthesia guidelines.
2. Monitor animal post-operatively for signs of distress or discomfort: abnormal posture or movement, lack of appetite, increased attention to surgical site. Contact LARC if there are recurring problems with recovery.
3. Administer post-operative analgesics per the IACUC-approved protocol.
4. Clean and dry all surgical instruments and re-assemble them to be sterilized again. Dispose of all soiled drapes, pads, towels, etc. Place all sharps in sharps disposal container.

5. Minimum required documentation: animal/cage ID, date, anesthetic and analgesic agents and doses, surgical procedure performed, and any surgical or anesthetic complications. If the protocol describes other parameters that will be recorded, these must be documented in addition to the above records.

6. Post-op animals should be monitored at least once daily for 3 days for any signs of discomfort, decreased appetite, or delayed wound healing. Document any problems and consult with the veterinarian if complications recur.

7. Remove wound clips or skin sutures 10-14 days post operatively. Any exceptions must be described in the IACUC-approved protocol.

In case of emergency, contact the veterinarian at XXX-XXXX.

Section 2 - NON-SURVIVAL (TERMINAL) SURGERY

Any surgery conducted on animals that are not allowed to regain consciousness is considered non-survival surgery. This includes terminal vascular perfusion.

- Non-survival surgeries require neither aseptic technique nor dedicated facilities, provided that animals are not anesthetized long enough to develop infections.
- Non-survival surgeries not performed aseptically or in a dedicated facility must at least be performed in a clean area, free of clutter. Personnel present in the area must observe reasonable cleanliness practices for both themselves and the animals.
- No expired drugs or fluids are allowed. Pharmaceutical-grade agents (USP) must be used unless an exemption is approved by IACUC.
- The IACUC must approve monitoring parameters for this type of surgery. The protocol must also describe the length of the procedure and steps taken to minimize the possibility of infection.
- The method of euthanasia should be consistent with the AVMA Guidelines on Euthanasia and must be listed in the approved IACUC protocol.
D) MAINTAINING ANIMAL STUDY AREAS (RODENTS)

For all animal procedure areas:

- The animal use space should be cleared of unnecessary equipment and clutter prior to bringing animals to the lab. All surfaces in the animal use space should be easily sanitizable. Materials made of wood, cardboard, or other materials that cannot be sanitized should be removed from the space.

- The fume hood or bench top should be cleaned prior to use with an appropriate disinfectant (i.e. chlorhexidine, bleach solution). Appropriate PPE should be put on before removing animals from cages.

- If live animals will be kept in a fume hood for any length of time, all chemicals should be removed from the hood.

- Maintain separate areas for cage storage, fur clipping, surgery, and non-surgical procedures, as each activity creates different levels of contamination.

- Supplies or equipment that come into contact with animals must be cleaned/disinfected before use with bleach, alcohol, or other appropriate disinfectant.

- Dirty cages are to be covered at all times and stored in a location that does not interfere with laboratory activities. All dirty cages should be removed from procedure areas as soon as possible to minimize allergen exposure. Cages must be removed from laboratory spaces by the end of the day, and should not be stored in the laboratory overnight.

- If animal use generates dirty bedding, feces, blood, or other contaminants, these must be cleaned up immediately.

- The animal use area must be disinfected immediately after animal work is completed. Cleaning the surface in between cages of animals is highly recommended to prevent cross-contamination.

- Inventory of expired drugs and supplies should be checked at least monthly. Expired materials must be discarded immediately or labeled “for in vitro use only” and stored in a non-animal use area.

For survival surgery areas:

- Aseptic surgery should be conducted in a dedicated facility or space. If an operating room is not available, an area physically separated from other laboratory activities may be acceptable.
• Surgical locations should have minimal traffic and contain surfaces that can be easily disinfected.

• The surgical preparation of the animal should be conducted in an area separated from where the surgery will be performed. The same table/bench top may be used for both surgical prep and surgery, but these areas must be distinct from one another.

• Always disinfect the surgery space prior to use, and between cages of animals. Non-sanitizable materials such as wood or cardboard should not be present in the surgery area.

• All surgical instruments and materials must be sterilized by steam autoclave or other appropriate means (refer to IACUC survival surgery guidelines) and kept sterile until they are used.

• Clean and disinfect surgery area immediately after use.

**For euthanasia areas:**

• Euthanasia equipment must be cleaned before and after use.

• It is preferable to euthanize animals in a hood, in their home cage if possible.

• Questions or comments: Please contact the Chair of the IACUC

**For Survival Surgeries**

Surgical preparation of the animal is performed in an area *separate* from where the surgery will take place.
E) EUTHANASIA GUIDELINES FOR RODENTS

General Guidelines
Euthanasia methods must be consistent with the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition, and be specified in the approved Institutional Animal Care and Use (IACUC) protocol.

- Use of an anesthetic agent for euthanasia must be administered at an overdose, not an anesthetic dose.

To confirm death, any chemical method used for euthanasia must be followed by a physical method from which the animal cannot recover, such as decapitation, exsanguination, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a major organ. The animal must be completely non-responsive to noxious stimuli (hind foot pad pinch on each foot) before any physical method is performed. All agents used are to be pharmaceutical grade.

- The techniques listed below are methods commonly approved in Saint Mary’s College of CA IACUC protocols for the euthanasia of rodents. Other methods outlined in the AVMA Guidelines on Euthanasia are acceptable when approved in the IACUC protocol.

A) Chemical Methods

Carbon Dioxide Inhalation/administration: CO₂ is delivered from a pressurized tank into an un-crowded cage to ensure precise regulation of gas inflow. The flow rate must be set to displace 10-30% of the chamber or cage volume/minute, allowing CO₂ to enter the chamber slowly so that unconsciousness and complete narcotization occur prior to death. Prefilled chambers are unacceptable. CO₂ flow should be maintained for at least one minute after respiratory arrest; animals must be left in the chamber for a sufficient time so that death has occurred prior to performing a physical method. When euthanizing mice, a standard size mouse cage may contain no more than 2 litters.

<table>
<thead>
<tr>
<th>Cage Type</th>
<th>Cage Size</th>
<th>CO₂ Flow Rate</th>
</tr>
</thead>
</table>

27
<table>
<thead>
<tr>
<th></th>
<th>CO2 Flow Rate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Supermouse750 (0.263 ft³)</td>
<td>2 L/min</td>
</tr>
<tr>
<td>Rat</td>
<td>Onecage2100 (0.970 ft³)</td>
<td>8 L/min</td>
</tr>
</tbody>
</table>

To ensure compliance with the AVMA Guidelines, a precision CO2 gauge/regulator with a pressure reducing valve or flow meter must be used. The table below provides the specifications for the CO2 flow rate for mice and rat euthanasia. Inspection and verification of flow rate that is in compliance with the AVMA Guidelines should be confirmed on an annual basis by the lab.

When possible, euthanize rodents in their home cage to minimize the stress of being placed into an unfamiliar enclosure and to prevent social aggression. Cages/containers used for euthanasia must allow clear visibility, be a size that permits full posture to be expressed, and be disinfected between uses to remove the potential distress that may be caused by exposure to remaining pheromone.
Injectable Anesthetic Overdose: Intraperitoneal injection of at least 200 mg/kg sodium pentobarbital is recommended; other injectable anesthetics may be approved and delivered at an overdose. Pentobarbital solutions can be viscous and are best diluted to a concentration of no more than 60 mg/ml. Intracardiac injections are suitable only if the animal is adequately anesthetized.

Inhalant Anesthetic Overdose: Isoflurane inhalation at an overdose may be utilized as a method of euthanasia, either by precision vaporizer or open-drop method. If open-drop isoflurane is utilized, it must be adequately scavenged to prevent personnel exposure. Animals may need to be exposed for prolonged time periods to ensure death.

Euthanasia while under anesthesia: When animals are fully anesthetized as at the end of a non-survival surgery, methods such as bilateral thoracotomy, exsanguination or perfusion are acceptable.

Temporary Holding Cages: On occasion, it may be useful for investigators to temporarily hold more than 5 mice per cage. For example: mice being collected for immediate euthanasia. This is acceptable as long as the following conditions are met:

- Up to 10 compatible mice may be placed in a temporary holding cage for up to 30 minute and holding cages are never left unattended.
- If fighting is observed, mice must be immediately separated.
- Adult males ≥ 6 weeks old from different cages should not be combined
- For mice and rat pups < 7 days old, refer to the below guidance

B) Physical Methods

Chemical methods must be followed by decapitation, exsanguination, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a major organ, and after the animal has been determined to be non-responsive to noxious stimuli.

Physical methods of euthanasia such as decapitation or cervical dislocation of un-anesthetized animals may be approved by the IACUC with appropriate justification in the IACUC protocol. The PI must ensure that personnel are experienced or properly trained.

Rats and Hamsters: Cervical dislocation may be acceptable if performed on animals less than 21 days and/or weighing less than 200 grams.

Guinea pigs: Cervical dislocation may not be performed on guinea pigs.

Fetuses and Neonates

- It is not necessary to remove fetuses for euthanasia after the dam is euthanized as they are unconscious in utero and hypoxia does not evoke a response.
• Inhaled anesthetics and CO2 are acceptable methods of euthanasia for neonates so long as adequate exposure time is provided (30-50 minutes for CO2 exposure) or an adjunctive physical method is performed after a neonate is nonresponsive to noxious stimuli.

• **Rats, mice, and hamsters less than 7 days old:** Rapid decapitation may be performed with sharp scissors or an adult decapitator, depending on tissue mass. It is recommended to remove all other live animals from the workspace when performing this procedure.

C) Maintenance and Use of Decapitation Equipment:

Equipment used for euthanasia of un-anesthetized animals such as commercial guillotines, scissors, or shears must be kept clean and serviced on a regular basis to ensure sharpness of blades. Clean and disinfect after each use. A final rinse with 70% ethanol will promote drying. Blades should be sharpened annually unless not in use, or more often as indicated. If returned to use, the blade(s) must be sharpened before first procedure.

The blades should be checked prior to each use for rust, cleanliness and ability to move freely without resistance. Dull blades should be replaced or can be sharpened by professional sharpening services. **Sharpening and maintenance records as well as sharpening SOPs need to be available during semi-annual inspections or upon IACUC request.**

Lab staff must be appropriately experienced or trained (see Physical Methods above). When using decapitation equipment, ensure hands and fingers are clear of blade path. The use of plastic restraint cones (i.e. Decapicones®) is recommended to restrain adult animals as they appear to reduce distress from handling, minimize the chance of injury to personnel and improve positioning of the animal in the guillotine.

Note: Guillotines should be periodically lubricated with silicone.

D) Disposal

ALL animal carcasses and tissues are treated as infected biohazardous waste and discarded in biohazard bags. The bags must be sealed and stored in closed waterproof containers with tight-fitted lids in designated cold rooms or freezers until removed by the animal waste management contractors. **Do not place the red bags in dirty cages being transported to the cage wash facility.**

E) Training: Only trained individuals may perform euthanasia. Training is provided in individual or group workshops through the IACUC Training and Compliance,
F) GUIDELINES FOR EUTHANASIA OF ZEBRAFISH

When possible, euthanasia should comprise a two-step process consisting of a chemical method followed by a physical method from which the animal cannot recover. The methods used must be specified in your approved IACUC protocol. Acceptable methods of euthanasia are described in the AVMA Euthanasia Guidelines under the Laboratory Finfish section.

Approved methods for adult zebrafish 7 days post fertilization (dpf) and older include:

- Tricaine (MS-222): Immers fish in a solution of tricaine methanesulfonate (Finquel or Tricaine-S). The solution should be buffered with sodium bicarbonate to a pH of 7.0-7.5. Fish must remain in the solution for 10 minutes following cessation of opercular (gill) movement.

- Rapid chilling: Submerge fish in 2-4°C chilled water. Fish should not be in direct contact with ice. Fish must remain in the chilled water for 10 minutes following cessation of opercular movement.

Zebrafish fry 4-7 days post fertilization:

- Tricaine or rapid chilling may be used as above, but fry should remain submerged in solution for 20 minutes following cessation of opercular movement

Zebrafish embryos 0-3 days post fertilization:

- Tricaine and rapid chilling are unreliable methods of euthanasia for embryos <3 dpf. Add dilute bleach solution (1 part sodium hypochlorite 6.15% to 5 parts water) to the water for 5 minutes to ensure embryonic lethality.

After one of the above methods has been performed, acceptable physical methods for all stages of development include maceration (for non-transgenic animals) or placement of the animal carcasses in the freezer.

References:
G) COHOUSING

The Guide for the Care and Use of Laboratory Animals (2011, National Academies Press, Washington, DC) recommends physical separation of different animal species for the following reasons:

- To prevent interspecies disease transmission
- To eliminate the potential for anxiety, physiologic and behavioral changes

However, the Guide does consider cohousing of species acceptable if the two species are of similar pathogen status and behaviorally compatible. The need to co-house animals of different species can be the result of several factors. These can include space limitations, research needs, sentinel requirements and/or equipment availability.

**Species Approved for Cohousing in the Same Animal Housing Room**

1. Rats and Mice
   - In all such cases, the animals have a similar pathogen status.
   - Animals are housed in individually ventilated cages

2. Gerbils and Hamsters
   - In all such cases, the animals have a similar pathogen status.
   - The animals are housed in individually ventilated cages.

3. Guinea Pigs and Mice
   - In all such cases, the animals have a similar pathogen status.
   - The animals are housed in individually ventilated cages.

4. Sentinel mice and other rodent species
   - A single cage of sentinel mice per rack may be cohoused in a housing room for other rodent species such as voles, hamsters, gerbils and guinea pigs.
   - Sentinel mice are tested on a quarterly basis and provide important information on the health status of the colony.

5. Different Species of Mice
   - Different species of mice such peromyscus or varying species of mice in the genus *Mus* may be cohoused in the same housing room.
   - In all such cases, the animals have a similar pathogen status.
   - These species are housed in individually ventilated cages or static microisolator cages.

6. Aquatic Animals
   - Turtles, frogs and salamanders may be housed in the same housing room.
   - Separation of each species occurs at the cage/tank level.

All nets and animal handling equipment remains separate between species.
H) RAT CAGE DENSITY

These recommendations are based on the guidelines provided in the Guide for the Care and Use of Laboratory Animals.

Cage Density
The following table indicates that maximum number of rats that may be housed in the rat cages:

<table>
<thead>
<tr>
<th>Body Weight of Each Rat (grams)</th>
<th>Static Cage (maximum # of Rats)</th>
<th>Ventilated Cage (maximum # of Rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 100</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>101 to 200</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>201 to 300</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>301 to 400</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>401 to 500</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 500</td>
<td>1-2*</td>
<td>2</td>
</tr>
</tbody>
</table>

*No more than 1000 g of total rat body weight is permissible in static rat cages

Enrichment and Social Housing
Rats must be provided with Environmental Enrichment. Provide at least one piece of enrichment in each cage (nestlet, shelter, additional nesting material, etc.).

Rats are to be socially housed. Singly housed rats are acceptable in the following situations and do not need separate IACUC approval: when they prove aggressive toward cage mates, are the last surviving member of a cage of animals, or when paired, maximum cage weight is exceeded. Other reasons must be based on scientific justification approved in the IACUC protocol.

Breeding
Monogamous Breeding is permitted. Ventilated rack cage capacity is one male, one female, and one litter. In static cages the male may not be housed in the same cage as a female and her litter.

- Cages are labeled with birth and P21 projected weaning dates
- Cages containing a litter older than P21 cannot also contain a newborn litter
  - For the welfare of a newborn litter, if two litters are present, then the new litter is transferred to a new cage.

Weaning
Litters are to be weaned at P21 and separated into same sex cages

Exceptions to this policy must be submitted in the IACUC protocol and approved prior to implementation.
I) MOUSE CAGE DENSITY

These recommendations are based on the guidelines provided in the Guide for the Care and Use of Laboratory Animals.

Cage Density
Maximum of 5 adult mice/cage are permitted in the standard 75in² mouse cages used in LARC.

Enrichment and Social Housing
Mice must be provided with Environmental Enrichment. Provide at least one piece of enrichment in each cage (nestlet, shelter, additional nesting material, etc).

Mice are to be socially housed. Singly housed mice are acceptable in the following situations and do not need separate IACUC approval: when they prove aggressive toward cage mates, the last remaining of a cage of animals, or mice removed from breeding cages. Other reasons must be based on scientific justification approved in the IACUC protocol.

Breeding
Monogamous Breeding (1M:1F) with one litter is permitted

- Cages are labeled by LARC with birth and P21 projected weaning dates
- Cages containing a litter older than P21 cannot also contain a newborn litter (For the welfare of a newborn litter, LARC staff is authorized to separate litters into new cages on a recharge basis when two generations of pups are present.)

Weaning
Litters must be weaned at P21 and separated into same sex cages.

For the occasional finding of small sized pups that may not be successfully weaned at P21, the cage must be labeled with the new expected weaning date and other litters may not be born into the same cage.

Exceptions to this policy must be submitted in the IACUC protocol and approved prior to implementation.
VIII) IACUC STANDARD PROCEDURES

A) OVARIECTOMY IN RATS AND MICE

1. The animal is weighed and anesthetized following the guidelines above. Surgical plane of anesthesia and peri-operative analgesia are required. Multimodal analgesia is encouraged.
2. The surgeon will follow IACUC guidelines for aseptic survival surgery.
3. Ovaries are typically approached by two separate flank incisions [Fig 1]. Alternatively, experienced surgeons may use a single midline skin incision on the back, if they can do this without excessive tissue manipulation [Fig 2]. Skin incision is approx. 5 mm in mouse and 10 mm in rat. Animal is kept in sternal recumbency if using a single incision, or laid on her side if using two incisions. The skin is separated from the underlying muscle before incising the muscle.

![Fig 1. Flank incision](image)

![Fig 2. Midline incision](image)

4. Before making the incision through the muscle overlying the ovary, the surgeon confirms the location of the ovarian fat pad, which is sometimes visible under the muscle. Rather than cutting the muscle, the tip of small double-sharp iridectomy scissors is inserted just through the muscle layer to separate the muscle fibers by opening the scissors in a dorsal ventral direction.

5. The ovary is gently pulled through the incision with a blunt forceps by grasping the fat pad surrounding it. A hemostat or similar is placed at the boundary between the oviduct.
and uterus, a ligature placed just below the hemostat (next to the uterus) and a cut is made just above the hemostat. Once the ovary and oviduct are removed, the hemostat is released and hemostasis is verified before letting go the uterus and allowing it to return to the abdomen. Muscle layer is closed, usually with absorbable suture. Skin is closed with suture or wound clips.

6. For the standard two-incision approach, the animal is turned over, and the second side treated as the first was done.

7. Anesthetic recovery follows the Saint Mary’s College of CA Anesthesia guidelines.
B) Vascular Perfusion with Fixatives for Tissue Collection

**Description of the Procedure:**

- This is a standard procedure for the vascular perfusion of anesthetized animals with fixatives for preparing tissues for histology. It is a non-survival surgery.

- Animals should be anesthetized following the Anaesthesia guidelines above.

- The usual route of perfusion is transcardial. Perfusion is usually done through the aorta or left ventricle. The right atrium is cut open. Animals are transcardially perfused through the ventricular catheter with saline followed by fixatives, or are directly perfused with fixatives. The perfusion solution drains through the incision in the atrium.

- Fixative solutions can also be infused through other large arteries and drained from an incision in a vein.

- Fixatives are usually infused under gravity or using a pump.

- The perfused animal is considered euthanized. Tissues are collected for analysis.
C) BAR HOLDING FOR GRIP STRENGTH

Description of procedure:

In order to assess limb strength and coordination, a mouse is allowed to hang by its forelimbs suspended from a wire or small bar.

Procedure Steps:

1. Padding is positioned under the bar so that the animal is not harmed upon releasing grip on the bar.
2. The mouse is allowed to hang by its forelimbs suspended from a wire of small bar. Mice should be able to hold on to the bar for 30-240 seconds.

Agents:

This procedure does not require any agents.
D) FORCED EXERCISE/WALKING WHEEL SYSTEM

Description of procedure:

The forced exercise/walking wheel system is a test that can be used for physiologic studies as well as activity based restorative therapies. The wheels incorporate a swing-hatch system for easy animal loading and removal. The hand held LCD interface permits a single exercise time and number of cycles. The animals will be loaded on the wheel in sequence. The exercise program consists of training rats to run on a motorized wheel system for up to 7.0 meters/min. 7.0 meters/min. is well below the exercise tolerance level in rats.

Supplies:

- Harvard Instruments with LCD monitor.

Procedure Steps:

1. The initial two-week training period involves a training period in which mice are put into the training wheel and put on the exercise bed and forced to run at a certain speed, for a certain amount of time.

2. The initial exercise speed will be 2.5 meters/min. for one hour.

3. The speed is then changed incrementally up to 7.0 meters/min for up to one hour.

Agents:

This procedure does not require any agents.

Adverse Effects:

<table>
<thead>
<tr>
<th>Procedure, Agent or Phenotype</th>
<th>Potential Adverse Effects</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Monitoring Parameters:

<table>
<thead>
<tr>
<th>Monitoring Parameters</th>
<th>Frequency</th>
<th>PI/Lab will Document</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity, Excreta, Grooming</td>
<td>During experimental manipulations (1-2 times)</td>
<td>Yes, if abnormal</td>
</tr>
<tr>
<td>Appearance, Behavior, Posture</td>
<td>Daily, during experiments</td>
<td>Yes, if abnormal</td>
</tr>
</tbody>
</table>
E) CROSSING ZEBRAFISH

Objectives: Fish are bred in order to generate embryos for experiments and for maintenance of the breeding stock. Transgenic fish are bred in order to generate animals carrying transgenes for labeling or genetic manipulation and genetic crosses are performed to produce particular genotypes and phenotypes.

Description of Procedure:

Cross adult fish at a ratio of 1 male to 1 female fish in breeding tanks overnight at a density of 4 or less fish per liter of water. The fish will be maintained in a crossing tank consisting of an upper (breeding) and lower (embryo collection) chamber separated by plastic or wire mesh.

Embryos from successful crosses will be collected the following morning. After crossing, adult fish will be returned to system tanks. Fish will be allowed to remain off system for no longer than 20 hours.

Fish will be allowed a minimum 2 week recovery period between productive crosses. Fish that have been set up without success will be allowed a 2 day recovery period before the procedure is performed again.

Potential Adverse Effects: There are no expected adverse effects associated with the procedure however the phenotype of the cross should be taken into account.
**F) OOCYTE COLLECTION FROM XENOPUS LAEVIS FROGS**

**Description of the procedure:**

Oocytes may be harvested up to six times per animal, alternating ovaries for each procedure. These procedures must be performed at least four weeks apart, with the last harvest being a terminal procedure.

**Pre-procedural preparation and anesthesia:**

- Clean gloves moistened with water should be worn at all times when handling frogs. All surgical procedures must be recorded and records checked to assure that the frog is an appropriate surgical candidate.
- Choose a female frog that is large and active. Transport frogs in water from their home tank to and from the lab. Collect the frog with a net that is dedicated to the tank.
- Anesthetize the frog with a fresh mixture of Tricaine methane sulfonate (MS222): 500mg-2g/liter dissolved in deionized water. The solution must be buffered.
- Submerge the frog in the MS222 and wait for the onset of anesthesia - it usually takes approximately 15-20 minutes.
- A surgical level of anesthesia is confirmed by gently pinching the fleshy part of both rear feet with hemostats or forceps and ensuring that the frog is non-responsive to painful stimuli.
- Place the frog on a clean surface.
- Remove contaminants on the skin by gently swabbing or spraying dilute surgical disinfection solution (chlorhexidine, povidone) only on the portion of the skin where the incision will be made.

**Oocyte Collection:**

- *The use of sterile instruments is standard practice for survival collection surgeries.* 'Cold' sterilization fluids (such as cidex or zephrin) should be avoided, as they may introduce potentially toxic chemicals into the surgical site or onto permeable amphibian skin.
- Make a small incision (1-2cm horizontal or vertical) on the abdomen above the groin and in between the midline and the lateral aspect of the abdomen.
- Blunt scissors are used to dissect through the fascia and muscle to visualize the oocytes. Oocyte strands are then gently externalized and cut.
- The incision is closed by suturing both the fascia and skin layer - it is recommended to swab the incision site with local anesthetic such as bupivacaine before suturing. Skin should be closed using an interrupted suture pattern to prevent dehiscence.
- Recover the frog in tank water with head elevated (to prevent drowning) and the rest of the body submerged. Recovery takes up to 1 hour. MONITOR FROG FREQUENTLY (no less than once every 10 minutes).

**Post-collection Care:**
• Frogs may be returned to a separate recovery tank in standard housing when animals are able to swim normally.
• All post-surgical animals must be appropriately identified. If there are multiple post-op animals in the same tank, then each animal must be individually identified (no-absorbable suture placed in webbing, web notching, etc.).
• Monitor frogs daily for a minimum of 48 hours for wound dehiscence or infection and then every 2-3 days for two weeks. Contact the LARC Veterinary Services group if there are any health concerns.

**Documentation required:**
• Identification of method used and location on the animal.
• Date of surgery, and applicable comments (e.g. frog recovered normally from anesthesia).
• Date of suture removal.
• Euthanasia.

**Agents:**
The procedure requires anesthetic MS-222. Analgesic (bupivacaine) is recommended. All agents must be listed on Section I of the protocol.

**Potential adverse effects to be considered:**
Infection of surgical site, dehiscence