Montana State University-Bozeman

IACUC Policies

Approved and Adopted by the Institutional Animal Care and Use Committee on: 09April2014
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose</td>
<td>4</td>
</tr>
<tr>
<td>Administrative Policies</td>
<td>4</td>
</tr>
<tr>
<td>Adoption</td>
<td>4</td>
</tr>
<tr>
<td>Administrative Change Policy</td>
<td>5</td>
</tr>
<tr>
<td>Animal Use According to Protocol Content</td>
<td>6</td>
</tr>
<tr>
<td>Animal Transfers</td>
<td>6</td>
</tr>
<tr>
<td>Congruency between Grants to Protocols</td>
<td>6</td>
</tr>
<tr>
<td>Full Committee Review Process</td>
<td>6</td>
</tr>
<tr>
<td>Guidelines on Prompt Reporting to OLAW under the PHS Policy</td>
<td>7</td>
</tr>
<tr>
<td>Non-pharmaceutical Grade Compounds</td>
<td>7</td>
</tr>
<tr>
<td>Non-response on Protocols and/or Modifications Pending Approval</td>
<td>8</td>
</tr>
<tr>
<td>Post-approval Monitoring (PAM)</td>
<td>8</td>
</tr>
<tr>
<td>Protocol Modifications</td>
<td>10</td>
</tr>
<tr>
<td>Reporting Animal Concerns (Whistle-Blower Policy)</td>
<td>10</td>
</tr>
<tr>
<td>Research Projects using Insects</td>
<td>11</td>
</tr>
<tr>
<td>Research Projects using Chick Embryos</td>
<td>11</td>
</tr>
<tr>
<td>Tours, Videotapes, Photographs and Cellular Phones</td>
<td>11</td>
</tr>
<tr>
<td>Training Policy</td>
<td>11</td>
</tr>
<tr>
<td>Vivarium Access</td>
<td>12</td>
</tr>
<tr>
<td>Animal Procedure Policies</td>
<td>12</td>
</tr>
<tr>
<td>Access to Nonhuman Primate Areas</td>
<td>12</td>
</tr>
<tr>
<td>Daily Checks</td>
<td>13</td>
</tr>
<tr>
<td>Escaped Rodents</td>
<td>13</td>
</tr>
<tr>
<td>Euthanasia</td>
<td>13</td>
</tr>
<tr>
<td>Enrichment Policy</td>
<td>15</td>
</tr>
<tr>
<td>Experimental Allergic Encephalomyelitis (EAE)</td>
<td>16</td>
</tr>
<tr>
<td>Food and Water Restriction</td>
<td>19</td>
</tr>
<tr>
<td>Humane Experimental Endpoints</td>
<td>20</td>
</tr>
<tr>
<td>Maximum Dosing Volumes</td>
<td>21</td>
</tr>
<tr>
<td>Non-terminal Blood Withdrawal</td>
<td>21</td>
</tr>
<tr>
<td>Physical Restraint</td>
<td>21</td>
</tr>
<tr>
<td>Polyclonal Antibody Production</td>
<td>22</td>
</tr>
<tr>
<td>The Use of Complete Freund’s Adjuvant in Rabbits</td>
<td>22</td>
</tr>
<tr>
<td>Sanitation Frequency of Primary Enclosures for Rodents</td>
<td>23</td>
</tr>
<tr>
<td>Single Housing of Rodents</td>
<td>24</td>
</tr>
<tr>
<td>Single Housing of Large Animals (Livestock)</td>
<td>24</td>
</tr>
<tr>
<td>Stabilization Period after Animal Transport</td>
<td>26</td>
</tr>
<tr>
<td>Tail Snips</td>
<td>26</td>
</tr>
<tr>
<td>Animal Transportation</td>
<td>26</td>
</tr>
<tr>
<td>Unexpected Outcomes</td>
<td>27</td>
</tr>
<tr>
<td>Xenopus Frogs</td>
<td>28</td>
</tr>
<tr>
<td>Zebra Fish</td>
<td>28</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Surgery and Anesthesia Policy</td>
<td>29</td>
</tr>
<tr>
<td>Anesthetic Cocktail Expiration</td>
<td>29</td>
</tr>
<tr>
<td>Avertin (Tribromoethanol)</td>
<td>29</td>
</tr>
<tr>
<td>Multiple Survival Surgeries</td>
<td>30</td>
</tr>
<tr>
<td>Pain Management</td>
<td>30</td>
</tr>
<tr>
<td>Rodent Surgical Guidelines</td>
<td>30</td>
</tr>
<tr>
<td>Survival Surgery</td>
<td>31</td>
</tr>
</tbody>
</table>
Purpose

This document describes the IACUC policies for research, teaching, and testing involving vertebrate animal species at Montana State University-Bozeman. These policies are in compliance with federal statutes and guidance from regulatory and accreditation agencies: Animal Welfare Act¹, USDA-APHIS², Public Health Service (PHS)³ and National Institutes of Health/Office for Laboratory Animal Welfare (OLAW), and AAALAC International.

Administrative Policies

Adoption

Background
The Animal Resource Center and Montana State University recognizes the invaluable contribution of research animals in the advancement of biomedical knowledge. The Institutional Animal Care and Use Committee (IACUC) endorses and promotes the responsible, humane, and appropriate use of research animals while complying with the concept of “refinement, reduction, and replacement” in our use of research animals.

The IACUC also recognizes that sometimes animals finish a research or teaching project in good health but are not suitable or needed for any other projects at the institution. In such circumstances, the Animal Resource Center may consider finding adoptive homes for the animals. It is understood and widely accepted that adoption programs not only enhance the quality of life for healthy research animals that are no longer needed by the University, but can also decrease stress and raise morale for both the research and animal care teams. We believe that responsible and compassionate researchers utilize animals only when necessary; therefore it follows that we should do our best to ensure good lives for those animals whose sacrifice is not required by science.

Purpose
The purpose of this policy is to allow research animals that are no longer assigned to protocols to be adopted by individuals as companion pets. The IACUC recognizes that adoption of research and teaching animals raises the potential for human health and safety concerns. While it is impossible to reduce health and safety risks to zero, animals selected for adoption must be healthy and present minimal risk of transmitting zoonotic infection.

Procedure
The following conditions must be met in order to place an animal for adoption:

1. Adoption privileges are granted only with the approval of the Attending Veterinarian.

2. An animal will be considered for adoption only if:

¹ 9 CFR Chapter1, Subpart A, sections 1, 2, and 3
³ PHS Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals, 8th edit. 2011.
a. The purpose for which the animal was acquired no longer exists 
b. The animal will not be needed in another IACUC approved study 
c. The animal has been returned to the Animal Resource Center 
d. An adopter has been identified to adopt the animal.

3. An animal may be placed for adoption only if considered clinically normal. 
   a. An animal must not have experienced any manipulation that has detrimentally 
      altered its anatomical, physiological, metabolic, or locomotor function. 
b. The animal must be free of detectable infectious disease or physical abnormality, 
   which would threaten animal or public health.

4. Before being released for adoption, animals must be examined by the Attending 
   Veterinarian and found to be in good health and of suitable temperament appropriate for a 
   pet. Cats and rabbits must be neutered and receive all age-appropriate vaccinations, as is 
   accepted by standard veterinary practice. Cats may be declawed if requested by the 
   adopting party with the understanding that the cat will remain an indoor pet

5. The individual adopting an animal must sign a waiver which states the University is not 
   liable for any injury or damage to persons or property by the adopted animal. The waiver 
   further states that no warranties, guarantees, or promises of any kind have been made or 
   can be made with regard to the adopted animal’s physical condition or temperament. The 
   owner assumes all further responsibilities associated with responsible companion animal 
   ownership.

6. An animal purchased from a Class A dealer (purpose-bred) may be eligible for adoption 
   as long as the above conditions are met.

7. At the time of transfer of ownership, the adopter shall assume all financial responsibility 
   for housing, care, and medication of that animal.

8. For each animal being adopted an “Animal Adoption Agreement” will be completed and 
   signed by the attending veterinarian and the adopting party. This record should be 
   maintained by the Animal Resource Center for a period of 3 years from the date of 
   signature by the new owner.

9. Any deviations from the provisions of this policy must be approved by the Director of the 
   Animal Resource Center and a simple majority of a quorum of the IACUC.

**Administrative Change Policy**

Changes that are not considered significant by the IACUC can be changed administratively by the 
program coordinator once the conditions for approval are met. Examples of such changes include:

1. Removal or addition of personnel, provided personnel meet the requirements for 
   IACUC approval (qualifications and training).
2. To correct spelling or similar typographical errors.
3. To correct technical errors that may arise from the protocol form.
4. To modify titles of existing protocols to meet requirements of funding agencies.
5. To add or delete funding sources.

Administrative changes will be documented in writing to the PI and the PI must review and 
approve of these changes.
Animal Use According to Protocol Content
Animals ordered for a particular protocol must be used according to methods reviewed and approved by the IACUC for that protocol. Animals may be transferred between protocols following the “Animal Transfers” Policy.

Animal Transfers
The transfer of animals to other protocols is allowed if the animal’s condition is satisfactory and the animal has not undergone or will not undergo surgery. The Animal Transfer Request form must be completed and submitted to the ARC. If procedures have been performed on more than one protocol, the animal use will be accounted for under each protocol.

Congruency between Grants to Protocols
The MSU IACUC attempts to confirm congruency between PHS grant applications and IACUC protocols through three methods. First, it is the Primary Investigator’s responsibility to provide correct and accurate information to both the IACUC and the funding agency. As part of the animal care and use protocol form, the PI must sign an Assurance and Statement of Accuracy. They must certify that the information is complete and accurate and consistent with any submission to external funding agencies. Second, prior to grant submission, the MSU Office of Sponsored Programs requires the investigator to submit a Proposal Clearance Form. If the grant includes the use of animals, either the IACUC Chair or the Attending Veterinarian must review and sign off on the application. Third, the Director of the Office of Sponsored Programs is a member of the IACUC, further assisting in the comparison of these documents.

Full Committee Review Process
Protocols are reviewed by the full IACUC at each monthly meeting. The committee Chair selects a committee member (Primary Reviewer) to take responsibility for describing the proposal and answering questions about the proposal at the full committee meeting. Protocols are sent to committee members a week before the meeting so each member has time to review the proposal before presenting the project to the committee. The Primary Reviewer is responsible for contacting the Primary Investigator prior to the meeting if protocol clarifications are needed. The Veterinarian and Chair also perform a “pre-review” prior to distributing the protocol to the Committee. Protocols require approval by a majority vote of a quorum of the full committee.

The committee may review and approve, require modifications to secure approval, or withhold approval if significant changes are required. If modifications are required to secure approval, the questions are submitted to the Investigator via email. The response is reviewed by the Primary Reviewer, the IACUC Chair and the Attending Veterinarian. If the response is unanimously approved by this designated subcommittee, it is added to the protocol file and final approval is granted. If one or more subcommittee member(s) are not satisfied with the response, it returns to the full committee at the next meeting.
Guidelines on Prompt Reporting to OLAW under the PHS Policy

The MSU IACUC will follow the Guidelines issued by OLAW, Office of Extramural Research on February 24, 2005. This guidance is intended to assist IACUCs and Institutional Officials in determining what, when, and how situations of noncompliance should be reported under IV.F.3 of the PHS Policy on Humane Care and Use of Laboratory Animals. PSH Policy, IV.F.3 requires that:

- “The IACUC, through the Institutional Official, shall promptly provide OLAW with a full explanation of the circumstances and actions taken with respect to:
  - Any serious or continuing noncompliance with this Policy;
  - Any serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals; or
  - Any suspension of an activity by the IACUC.”

These guidelines are available at:

Non-pharmaceutical Grade Compounds

Investigators are expected to use pharmaceutical-grade compounds whenever they are available\(^4\),\(^5\),\(^6\),\(^7\),\(^8\) to avoid toxicity or side effects that may threaten the health and welfare of the animals and/or interfere with the interpretation of research results. Non-pharmaceutical grade compounds should only be used after specific review and approval by the IACUC of the written justification for use in an animal protocol.

Scientific justification for the use of non-pharmaceutical compounds may include the following reasons\(^9\):

- non-availability of an equivalent veterinary or human drug
- specific exception to an available veterinary or human drug, for example:
  - insufficient strength of the active compound in an available formulation
  - presence of an excipient or preservative is unacceptable for proposed studies (e.g. toxic via planned administration route, nature of excipient would affect experimental model or compromise data collection)
  - use of an available formulation requires further change (e.g. dilution or other addition) and offers no advantage over formulation from a high-quality reagent
- scientific necessity for comparability to previous research or to replicate specific experimental model

In addition to the scientific justification for use, the appropriate chemical properties of a non-pharmaceutical grade compound should be considered by the Investigator for the proposed study and route of administration. The grade/purity, potency, concentration, pH, osmolality, stability,

---

\(^4\) 9 CFR Chapter1, Subpart A, sections 1, 2, and 3
\(^5\) US Department of Agriculture /Animal and Plant Health Inspection Service - Animal Care Policy #3 Veterinary Care, March 25, 2011.
\(^6\) PHS Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals, 8th edit. 2011.
\(^7\) Office of Laboratory Animal Welfare: LAW Position Statement: Non-Pharmaceutical Grade Substances: http://grants.nih.gov/grants/olaw/positionstatement_guide.htm#nonpharma
\(^8\) Association for Assessment and Accreditation of Laboratory Animal Care International, FAQ http://www.aaalac.org/accreditation/faq_landing.cfm/#B9
\(^9\) Cost savings is not included as an appropriate justification for use.
formulation (buffer or solvent), and potential contaminants (e.g. chemical, biological, and microbial, including pyrogenic substances), as well as handling and storage procedures, are among the properties and practices that impact quality of the compound for achieving the scientific aims of the study.

Definitions

- **Pharmaceutical grade compound** - an active or inactive drug, biologic or reagent for which a chemical purity standard has been established by any recognized pharmacopeia, such as the: US Pharmacopeia (USP)/National Formulary (NF), British Pharmacopeia (BP), or Pharmacopoeia of the Council of Europe (EP). These include, but are not limited to, pharmaceutical compounds approved for human or veterinary use approved by the U.S. Food and Drug Administration (FDA).

- **Chemical Purity Grades** – the majority of chemicals are manufactured to comply with the International Organization for Standardization (ISO) regulation ISO 9001:2008, and laboratory chemicals manufactured to standards set by the American Chemical Society (ACS).

- **Biologics** – biological molecules, obtained either by collection or extraction and purification from living systems, or by production in recombinant expression systems, or by de novo chemical synthesis. In terms of FDA licensed products, examples include antitoxins, antivenins, blood, blood derivatives, immune serums, immunologic diagnostic aids, toxoids, vaccines, and related articles.

- **New Investigational Compound** - supplied by its manufacturer for testing in an experimental setting only and for this reason would not have chemical purity standards established; by default is considered a non-pharmaceutical grade compound.

Other Resources

- c. FDA Drug Approvals and Databases including inactive ingredients search, the Orange Book (human drug products) and the Green Book (animal drug products, at URLs: [http://www.fda.gov/Drugs/InformationOnDrugs/default.htm](http://www.fda.gov/Drugs/InformationOnDrugs/default.htm) and [http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/default.htm](http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/default.htm)
- d. 21 CFR Parts 580 and 582. Substances generally recognized as safe (GRAS). [http://www.ecfr.gov/cgi-bin/ECFR?SID=78cbb875ff401e5455563e0b7cd1b049&page=browse](http://www.ecfr.gov/cgi-bin/ECFR?SID=78cbb875ff401e5455563e0b7cd1b049&page=browse)

**Non-response on Protocols and/or Modifications Pending Approval**

If there are questions on a submitted protocol and/or modification and the PI fails to respond within 60 days of written documentation, the protocol and/or modification will be administratively withdrawn and require a new submission.

**Post-approval Monitoring (PAM)**

The Montana State University post-approval monitoring (PAM) program is in place to ensure the well-being of animals involved in research activities, to make certain that activities are conducted in compliance with state and federal regulations and standards, and to ensure that activities are consistent and conducted in compliance with IACUC approved protocols, as well as other institutional policies.
The IACUC is ultimately responsible for performing post-approval monitoring. A multitude of individuals contribute to the post-approval monitoring program, including the IACUC, the research staff, the ARC staff, and the Attending Veterinarian. A variety of procedures are in place to assure compliance. These include:

- The research program and facilities are reviewed on a semi-annual basis. Any issues of noncompliance are monitored for appropriate follow-up by the IACUC by at least two voting members.
- Through initial orientation and periodic continuing education, all personnel involved in animal studies are aware of their responsibilities as they relate to animal activities. Protocols are available for review and all research personnel are expected to be familiar with the protocol under which they are working.
- Primary Investigators receive annual training to explain new regulations and ensure compliance with federal statutes and guidance from regulatory and accreditation agencies. In addition PIs receive instruction on protocol submission and are made aware of all program changes, e.g., updates to protocol form, including new questions.
- Animals are housed under centralized conditions. All mammals are housed either at the Animal Resources Center or the Jutila Research Laboratory. The ARC staff is responsible for routine daily observations and monitoring of animal research activities. The ARC Manager and four of the staff members are AALAS certified at the LATg level. In addition, they are involved in assisting research staff with many of the research procedures and they communicate directly with the research staff on a daily basis. Each ARC staff member rotates through the IACUC on an annual basis and the Director and Manager have access to all currently approved protocols.
- All animal ordering is performed centrally by the ARC. A database is maintained which tracks the number of animals approved for each protocol and the number of animals ordered. This record-keeping system does not allow personnel to go beyond the number of animals approved on a protocol. If the investigator is close to the maximum number of animals allowed, an informational email is sent.
- All in-house breeding colonies are centrally maintained by the ARC staff. Animal numbers produced by the breeding colonies are counted at the time of weaning and an “Animal Transfer Request” form is submitted. These numbers are entered into the database in the same way as animals ordered from outside sources.
- The animal facility can be accessed only through the use of a keycard. This is used to limit access to individuals who are approved to work with animals. There are different access levels, ABSL1 access, ABSL2 access, and ABSL3 access.
- Laboratories where animals are held longer than 12 hours (USDA covered species) or 24 hours (mice and rats) are reviewed as part of the semi-annual IACUC inspections.
- MSU has adopted the 2013 AVMA Guidelines on Euthanasia. All CO2 euthanasia stations have been fitted with flow meters to displace 10 – 30% of the chamber or cage volume/minute. Laboratories that utilize CO2 as a method of euthanasia are inspected for proper use and training is provided for all users.
- MSU is small enough that we are able to maintain appropriate and short organizational reporting chains. The Attending Veterinarian and IACUC works closely with the Institutional Official on animal welfare and compliance issues/concerns.
• A whistle-blower policy is in place to assure noncompliant issues are reported and animal welfare issues are resolved.
• Annual renewals of protocols are reviewed by the full IACUC.

Protocol Modifications
Any proposed modifications of animal use methods must be communicated to the IACUC. Major modifications will be reviewed and approved by the full committee prior to implementation. Examples of major modifications include the following changes:

- The objectives of the study
- Non-survival to survival surgery
- Increasing the invasiveness of a procedures or pain/distress to the animal
- Change or addition of species
- Increasing the number of animals used
- Anesthetic or analgesic agents
- Method of euthanasia
- Status of biosafety, radiation safety, or environmental safety usage
- Change in the Primary Investigator

Reporting Animal Concerns (Whistle-Blower Policy)
The use of animals in biomedical research is necessary and appropriate. Montana State University is committed to the humane treatment of animals and strives to ensure that institutional facilities and procedures adhere in all respects to NIH guidelines for care and use of laboratory animals.

The IACUC wants to be informed of any concerns that individuals of the campus community may have with respect to the care and use of laboratory animals and feels it is important that these concerns be addressed on an individual basis.

Any individual who witnesses what he/she perceives to be inappropriate treatment of research animals has several options to report the inappropriate treatment.

a. Contact any member of the IACUC and report the concern.

b. File a concern by filling out an “Animal Concern Form” (posted at all locations where animals are housed), which will be submitted to the IACUC.

c. MSU has instituted an anonymous Compliance Hotline via a third party company, Ethics Point. A report can either be made via phone or through an online reporting system that allows reporter to remain anonymous. The link is: https://secure.ethicspoint.com/domain/media/en/gui/38620/index.html and the phone number is 855-753-0486.

Every effort will be made to keep the identity of the person raising the concern confidential. Retaliation against a person who raises a good-faith concern will not be tolerated. The IACUC will follow-up on all complaints and gather additional information as necessary and appropriate. If indicated, corrective action will be initiated and the results of the investigation will be communicated to the individual who raised the complaint.
**Research Projects using Insects**
Protocols utilizing insects are exempt from IACUC review.

**Research Projects using Chick Embryos**
Protocols utilizing chicken embryos up to day 15 of incubation are exempt from IACUC review.

**Tours, Videotapes, Photographs and Cellular Phones**
Tours of the ARC must be approved by the ARC Director or Manager. Requests will be reviewed on an individual basis, and if questionable, will be referred to the IACUC for approval. Any request for videotaping or photographing animals and animal procedures need to be approved by the Attending Veterinarian or IACUC. In addition, once approved, the AV or IACUC can select to review the finished product before its release or publication. Cell phone use in the ARC is highly discouraged and should be used only for essential professional communications. In ABSL1 areas, the cell phone should be considered a potentially contaminated fomite and should not come in contact with any item that comes in contact with the animals (e.g. gloves). Cell phones should not be used within the ARC ABSL2 areas or the JRL.

**Training Policy**

**Animal Care and Use Training Requirements**
Federal regulations require that all individuals handling vertebrate animals receive training about animal use in general, federal law regarding animal use, species specific handling, as well as in the specific procedures necessary for their experiments.

All personnel at Montana State University who work with research animals must perform the following training prior to gaining access to the animal facility.

1. A three-hour introductory training session is mandatory for anyone working with small laboratory animals. The sessions are taught by the ARC Director, Occupational Safety Officer and Facility Manager. The Director covers topics such as regulatory compliance, protocol requirements, the Three Rs, methods for reporting concerns about animal use, animal welfare and pain and distress. The Occupational Safety Officer covers topics such as allergies, ergonomics, and university medical screening requirements. The Manager discusses ARC policies related to facility security and animal care.

2. The lecture portion of the course is followed by a hands-on wet lab that demonstrates proper handling and restraint, as well as basic technical procedures (injections, blood collection, etc.) in mice.

3. Prior to being granted access to the animal facility, personnel must also take a web-based course offered through CITI.

4. Once they have completed both the training session and the on-line course, they are given a proximity reader for access to the facility.

**Personnel working with nonhuman primates:**

1. Those personnel working with nonhuman primates must attend an additional training session covering safety, risks, and zoonoses related to working with nonhuman primates. All new personnel must also view Griffin Foundation video
“Working safely with NHP’s”. A tour of the NHP housing area is included with a review of management and handling techniques and NHP behavior. Information about Herpes B virus is emphasized and the exposure kit is reviewed during the tour.

2. Prior to being granted access to the nonhuman primate housing area, personnel must also take a web-based course offered through CITI.

**Personnel performing surgical procedures:**

1. Personnel performing surgery must complete a training session taught by the ARC Director, covering aseptic technique.

The ARC Director also provides guidance and training regarding the proper use of anesthetics and analgesics.

**Principal Investigator (PI) Training**

Primary Investigators receive annual required training to explain new regulations and ensure compliance with federal statutes and guidance from regulatory and accreditation agencies. This training is completed as a condition of initial protocol approval as well as when the protocol is up for full protocol renewal every three years. In addition PIs receive instruction on protocol submission and are made aware of all program changes, e.g., updates to protocol form, including new questions via our IACUC Newsletter which will be posted on our website. The Office of Research Compliance will maintain written documentation once the training is completed.

**Vivarium Access**

Both the Animal Resources Center and the Jutila Research Laboratory require keycard access. For the ARC, research and animal care staff must attend the orientation session and complete the CITI on-line course for the appropriate species prior to gaining access to the facility. There are two levels of access that can be granted, and access given is dependent on the request of the PI in whose lab the person will be working. Access options include ABSL1 area only, and ABSL2 access. The Investigators are responsible for notifying the ARC Manager when someone is no longer working in the laboratory. At that time, the keycard is inactivated. All guests must be escorted in the facility by approved personnel who will ensure they follow all established policies and procedures.

For the Jutila Research Laboratory, individuals must complete select agent requirements and access must be granted through the MSU Biosafety Officer and the Laboratory Director.

**Animal Procedure Policies**

**Access to Nonhuman Primate Areas**

Personnel who require access to the nonhuman primate areas are required to attend a nonhuman primate orientation session administered by the Director of the ARC. They must also complete the CITI course on nonhuman primates. Personnel who enter nonhuman primate areas are required to wear a dedicated long-sleeved lab coat or disposable gown, gloves, disposable bonnet, surgical mask, shoe covers, and eye protection. Long pants are required and open-toed shoes are not allowed.
Daily Checks

The ARC staff performs a daily health check on all animals. For large animals (rabbits, monkeys, etc.) this involves direct visualization of each animal daily. For rodents this constitutes a “daily cage check” in which each cage is checked for food, water, cage flooding, deaths, births, or any obvious items that can be visualized without removing the cage from the rack or disturbing sleeping animals or litters. Direct visualization of each mouse occurs when the cage is changed (weekly or bi-weekly). Issues are reported to the Veterinarian, the Investigator and/or appropriate lab representative. These daily cage checks represent an acceptable and normative practice that meets the regulatory requirements of monitoring the health of research animals. ARC staff is not required to directly visualize or examine each mouse in every cage on a daily basis as this would be impractical given the number of animals and in the case of breeding colonies, could be disruptive of ongoing research.

The research personnel identified on the approved protocol are responsible for monitoring the health of animals on study to the level indicated in the respective IACUC protocol. This monitoring is independent of the daily cage check performed by the ARC. The lab can request the services of the ARC to perform a more in-depth check of the animals, and is performed for a fee based on time required.

Escaped Rodents

If a rodent escapes within the hood during the process of cage cleaning or handling and can be easily identified, it is placed back in the appropriate cage. If a rodent escapes to the floor or is found loose in an animal holding room and cannot be positively identified, it is placed in a separate cage and isolated or taken to the necropsy room for euthanasia. If a rodent is found loose within the facility outside an animal room, such as in the feed and bedding storage area, cage wash room, etc. the animal is captured, placed in a separate cage and taken to the necropsy room for euthanasia. Prior to euthanasia, the ARC staff will perform a health analysis on the rodent similar to that done for sentinel animals. Blood will be collected for serology and the animal will be examined for external and internal parasites.

Euthanasia

When required for scientific data collection or clinical or operational requirements, animals will be humanely killed, as detailed in the approved protocol. Unintended recovery of animals after apparent death constitutes serious noncompliance with PHS Policy and serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. MSU is obligated to report to the NIH any instances of animals awaking after intended death.

Public Health Policy (PHS) requires IACUC’s to determine that methods of euthanasia proposed in protocols meet criteria outlined in the 2013 Report of the AVMA Panel on Euthanasia. Primary Investigators (PI) are expected to follow the methods of euthanasia as outlined and approved in their protocols. All individuals who may perform or be expected to perform euthanasia should be familiar with the details in the protocol and the information outlined in this policy. Training in proper methods of euthanasia is part of the initial orientation and is provided by the ARC Director and staff.

Acceptable methods for adult animals include: Carbon Dioxide (rodents only), Isoflurane, barbiturates, physical methods performed under anesthesia (cervical dislocation, exsanguination,
decapitation), and MS222 (tricaine methane sulfonate) for aquatic species. Physical methods of euthanasia without anesthesia are conditionally acceptable and must be reviewed and approved by the IACUC. Personnel responsible for performing these techniques must be properly trained.

**Carbon Dioxide Euthanasia**

The AVMA Panel on Euthanasia (2013) has stated that CO2 is an acceptable euthanasia agent for small laboratory animals, when used properly. The Panel states that the optimal flow rate for CO2 euthanasia systems should displace 10% to 30% of the chamber or cage volume/minute. The Attending Veterinarian and the Animal Resource Center staff are responsible for training in the use of CO2 for euthanasia. Research staff is required to follow these procedures and the IACUC is responsible for monitoring the proper use of such agents.

MSU Guidelines for using CO2 include:

1. Compressed CO2 in gas cylinders with flow meters is the only acceptable source of CO2 for euthanasia. CO2 generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g. antacids) is unacceptable.
2. Only animals of the same species should be placed into a chamber at any time.
3. Animals must be placed into chamber so that they have sufficient floor space and are not overcrowded.
4. Euthanasia of more than one animal at a time should always be performed in cohorts of live animals (i.e., live animals must never be placed in the chamber with dead animals).
5. To reduce stress, rodents should be euthanized in their home cage whenever possible.
6. Alternatively, if animal groups are combined, the time between combining groups and euthanasia should be kept to a minimum.
7. The AVMA Panel (2013) has stated that an optimal flow rate for CO2 euthanasia systems should displace 10% to 30% of the chamber or cage volume/minute, and that pre-filled chambers are unacceptable.
8. Bags are not acceptable chambers.
9. Chambers/lids must be cleaned and dried between animals or groups of animals to minimize odors that might distress animals.

**Verification of death:**

Once all animals have lost consciousness, CO2 flow can be increased and should be maintained for at least one minute beyond apparent clinical death (cessation of cardiovascular and respiratory movement). Death must be verified by assuring cessation of respiratory and cardiovascular movements after a minimum of 10 minutes exposed to room air, or by employing a secondary method of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy prior to carcass disposal.

**Fetal and Neonatal euthanasia (Rodents)**

The AVMA Guidelines on Euthanasia (2013) do not provide detailed recommendations for the euthanasia of prenatal and neonatal rodents. These guidelines are based on a combination of the AVMA Guidelines and NIH recommendations; Exceptions to these guidelines will be considered by the IACUC on a case-by-case basis.

- Rodent Fetuses:
• Fetuses up to 14 days in gestation: rodent fetuses are unconscious in utero and hypoxia does not evoke a response. Therefore, it is unnecessary to perform euthanasia on the fetus after euthanizing the dam.
• Fetuses 15 days in gestation to birth: Whereas fetuses at this age are less sensitive to inhalant anesthetics, euthanasia may be induced by the skillful injection of chemical anesthetics. Decapitation with surgical scissors or cervical dislocation is acceptable physical methods of euthanasia. Anesthesia may be induced by hypothermia of the fetus (no direct contact), by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g., pentobarbital. The veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.

- Rodent Neonates:
  • Up to 7 days of age: Gradual cooling is acceptable, but the animals should not come in direct contact with ice or precooled surfaces. Decapitation using scissors or sharp blades is acceptable. Training is required before this method can be instituted.
  • Up to 14 days of age: Acceptable methods include: injection of chemical anesthetics (e.g., pentobarbital) or cervical dislocation. Additionally, inhalant anesthetics may be used, however death must be confirmed by a secondary means. The veterinarian should be consulted for appropriate agents and dosages.
  • Older than 14 days: Follow guidelines for adults.

**Enrichment Policy**

Various terms are used to describe the welfare requirements of research animals, such as psychological well-being, environmental enrichment, behavioral needs, etc. The 1985 amendments to the Animal Welfare Act require facilities to provide exercise for dogs and programs to promote the psychological well-being of nonhuman primates. The Guide for the Care and Use of Laboratory Animals states:

“The primary aim or environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics.”

The ARC is responsible for the Enrichment Program. The mission is to enhance well-being of all laboratory animals housed within the MSU research program through appropriate housing, enrichment, health care and activity, thereby allowing the animals to engage in species-typical behaviors. Methods of enrichment include, but are not limited to: social housing; housing devices; manipulative devices; visual, auditory, and olfactory devices; food items. The goals of the program are to address the psychological well-being of all species housed in the facility, provide interaction and self-initiated behaviors by allowing a degree of control over their environment, and to increase species-typical behaviors while decreasing pathological maladaptive behaviors.
The complete and detailed program has been reviewed and approved by the IACUC and is available from the ARC.

**Experimental Allergic Encephalomyelitis (EAE)**

EAE (experimental allergic encephalomyelitis) is an autoimmune animal disease model of the central nervous system (CNS) mimicking some aspects of human multiple sclerosis (MS). Although clinical signs vary, they include visual, sensory and motor deficits. This generally manifests as an ascending paralysis graded on a five-point scale from loss of tail tone (1) to moribund (5). The course may vary from one or more episodes with short periods of remission of clinical signs to a progressive chronic state. This guideline describes the specialized care of EAE animals to ensure their humane care and treatment.

**Care:**
Every animal to be injected with any substance to elicit EAE should be identified with a cage card with the letters "EAE."

Animals should be monitored at least daily, including weekends and holidays.

Food should be put in the bottom of the cage and water should be accessible should partial paralysis begin to become evident. An alternative source of water should be provided (i.e., Napa nectar).

Monitoring will include the graded score of the EAE (Table 1) involvement, hydration status, general condition and activity level of the animal. All the observation and treatments must be recorded on a research record, dated and initialed.

All paralyzed mice should be monitored for skin irritation associated with urine scald and, if male, observed for penile irritation secondary to flaccid paralysis.

Remove animals before paralysis and singly house if they are housed with normal mice. Normal animals may walk on paralyzed animals causing discomfort or even injuries, and may eat the food intended for paralyzed animals.
Table 1. Grading system for clinical assessment of EAE

<table>
<thead>
<tr>
<th>EAE Grade</th>
<th>Behavioral &amp; Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal mouse; no overt signs of disease</td>
</tr>
<tr>
<td>1</td>
<td>Limp tail or hind limb weakness, but not both</td>
</tr>
<tr>
<td>2</td>
<td>Limp tail and hind limb weakness</td>
</tr>
<tr>
<td>3</td>
<td>Hind limb paresis</td>
</tr>
<tr>
<td>4</td>
<td>Four-limb paralysis</td>
</tr>
<tr>
<td>5</td>
<td>Moribund state; death by EAE; sacrifice for humane reasons</td>
</tr>
</tbody>
</table>

Table 2. Clinical Assessment of EAE: All mice will be evaluated daily by the investigator for signs of disease and graded on a scale of 0 to 5 according to the severity of the symptoms.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Clinical Sign</th>
<th>Intervention Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormality</td>
<td>Baseline weight (average/cage)</td>
</tr>
<tr>
<td>1</td>
<td>Initial signs but no paraparesis; clumsiness; incontinence; or atonic bladder</td>
<td>If atonic bladder present, express daily and check hydration status twice daily. At this time additional nutrients should be added to cage bottom. Initiate a medical record and an EAE chart and record weights.</td>
</tr>
<tr>
<td>2</td>
<td>Mild paraparesis; trouble initiating movement, but walk well once started; possible atonic bladder</td>
<td>Same as above; if five mice in a cage and only one affected, consider separating to allow better access to feed. Mice may be fed additional nutritional support as needed. Begin weighing animals three times per week and record weights on the medical record and the EAE chart.</td>
</tr>
<tr>
<td>3</td>
<td>Moderate paraparesis; inability to move one or both hind legs, noticeable gait disturbance, possible atonic bladder</td>
<td>Food and water more accessible (for example, feed mash placed on floor of cage, water assessable, and fruit as fluid supplementation). Express urinary bladder twice daily; give fluids, if necessary. Animals may need supplemental heat (see no. 4 below). Weigh at least three times per week. Euthanize if greater than 20% weight loss.</td>
</tr>
<tr>
<td>4</td>
<td>Moderate quadriparesis/quadriparalysis</td>
<td>Euthanize, except when investigators develop and implement a plan with the AV.</td>
</tr>
<tr>
<td>5</td>
<td>Moribund</td>
<td>Euthanize</td>
</tr>
</tbody>
</table>

Table 3: Treatment Procedures

<table>
<thead>
<tr>
<th>Problem</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>Lightly pinch the skin over the neck to determine skin turgor. If skin remains in “tented” position, administer 1ml normal saline or lactated ringer’s solution subcutaneously twice per day.</td>
</tr>
<tr>
<td>Urination</td>
<td>At least 2 times per day (morning and afternoon) and additionally as needed, roll the mouse on its back and lightly palpate the abdomen to assist with voiding.</td>
</tr>
<tr>
<td>Weight</td>
<td>Body weight must be measured as least three times per week</td>
</tr>
</tbody>
</table>

Endpoints:
EAE grade 4 (see Table 2 for exceptions) and 5
Body weight loss > 20%
Veterinarian recommendation

Any exceptions to the above policy must have IACUC approval.

Induction of EAE
EAE is a demyelinating CNS disease that serves as an animal model for Multiple Sclerosis (1, 2). EAE can assume an acute, chronic, or relapsing remitting disease course depending on the method of induction and type of animal used. EAE in general will assume a variable disease course that progressively results in degrees of ascending animal paralysis. The resulting paralysis is debilitating, but not painful and most animals will show some degree of recovery even from advanced stages of EAE. Paralysis typically begins with a weakened tail, followed by hind limb paralysis, and rarely front limb paralysis. Since EAE is a variable disease, there is no reliable way to predict whether an animal will recover. As a result, close monitoring is needed in this animal model.

EAE can be induced with components of the central nervous system (3, 4) or peptides (5,7) and also via T cell transfer from one animal to another animal (8). Complete Freund's Adjuvant (CFA) is used with the extracts or peptides and is often used in combination with pertussis toxin (9, 10). Pertussis toxin is thought to play a role in the breakdown of the blood brain barrier (11). Administration of analgesics to lessen pain associated with CFA injections is not possible since most analgesics affect the immune response that is an essential component of the model (12, 13).

This policy is intended for investigators using the EAE model, providing justifications and guidelines for the use of CFA, pertussis toxin, cell transfer, and monitoring of the animals.

Other Resources:


**Food and Water Restriction**

**Background:** Behavioral research often requires that an animal perform a task for which it receives food or fluid reward. This situation is not unlike conditions in the wild, in which animals must forage, travel distances, solve problems, or otherwise work to obtain food and water. Performing a task for rewards may also be behaviorally enriching for laboratory animals, especially nonhuman primates. However, a fundamental concern with studies that may involve food or water restriction is that animals are maintained in a healthy state and that they do not experience pain or distress. This document provides an overview of the issues and explains the IACUC’s policy regarding food or water restriction.

The IACUC referenced the following documents in setting this policy:

- The federal Animal Welfare Regulations (Code of Federal Regulations, Title 9).

**Definitions (Guide pg 30):**

- **Scheduled access:** the animal consumes as much as desired at regular intervals.
- **Restriction:** the total volume of food or fluid consumed is strictly monitored and controlled.
- **Fasting for surgical procedures:** usually a period of less than 12 hours. This is not considered restriction and the following guidelines do not apply.

**Policy:**

1. Individual animals may vary in their physiologic response to food or fluid restriction. Therefore, it is imperative that animals be closely monitored on a daily basis to ensure that they are healthy, adapting normally and consuming sufficient food and water to maintain their health status. Monitoring and, if necessary, early intervention are the most important objectives of this policy.

2. It is the obligation of the investigator to demonstrate to the IACUC that food or water restriction is the only effective way to accomplish the scientific goals of the study. The objective when these studies are being planned and executed is to use the least restriction necessary to achieve scientific objectives while maintaining animal well-being.

3. It is the investigator’s ongoing obligation to continue to attempt methods of positive reinforcement that do not involve food or water restriction and to use restriction only when other methods fail.

4. The use of restriction to motivate behavior must be specifically discussed, adequately justified, and approved in each protocol in which it is used.

5. Criteria for monitoring animal health must be defined in the protocol (i.e., body weight, urine and fecal output, urine specific gravity, etc.). Criteria must be defined for temporary or permanent removal of an animal from the experimental protocol.

6. Investigators must maintain water consumption and food intake records in a form acceptable to the IACUC and readily available for inspection.

7. At the discretion of the IACUC, investigators may be required to file updates or reports regarding the status of the animals on the restriction protocol.

8. At the discretion of the veterinarian, a particular animal’s food or water ration must be increased if:
   a. The animal becomes ill or requires medical treatment.
   b. The animal exhibits weight loss of greater than 10-15% of its initial body weight.
   c. The animal shows evidence of clinical dehydration.
   d. A young animal fails to show reasonable weight increase during a time when it should be growing.
   e. The animal undergoes any procedure requiring anesthesia.
   f. Under any other circumstance deemed necessary by the veterinarian.

### Humane Experimental Endpoints

The Guide for Care and Use of Laboratory Animals (8th Edition) defines the humane endpoint as the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress, including death.

The PI, who has precise knowledge of both the objectives of the study and the proposed model, is required to identify, explain, and include a study endpoint that is both humane and scientifically sound. The PI is also required to define the criteria for humane intervention for the study, listing how often the animals will be monitored to evaluate if they have reached the humane endpoint, and provide information on training of personnel performing the assessment.

When evaluating humane endpoints, the following specific sequela should be considered:
1. The procedure should not interfere with the ability of the animal to ambulate, eat, drink, urinate and defecate.
2. The procedure should not result in a net weight loss of more than 20% of the body weight.
3. The procedure should be ended if the investigator has conclusive evidence that untreatable organ failure has occurred, and the animal exhibits signs associated with the failure of the organ system.
4. Animals should be euthanatized if unrelated health conditions develop that make their experimental use of no value to the investigator.
5. Obvious signs of illness should serve as alternatives to death as an experimental endpoint.

The IACUC defines the moribund condition as a severely debilitated state that precedes imminent death. The use of death as an endpoint requires strong scientific justification in the Protocol and full committee review and approval by the IACUC.

### Maximum Dosing Volumes

<table>
<thead>
<tr>
<th></th>
<th>Adult Mice</th>
<th>Rats (&gt;250 grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intramuscular (IM)</td>
<td>0.1 ml</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>Subcutaneous (SC)</td>
<td>3.0 ml</td>
<td>10 ml</td>
</tr>
<tr>
<td>Intraperitoneal (IP)</td>
<td>2 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>Intravascular (IV)</td>
<td>0.25 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>Oral (PO)</td>
<td>0.5 ml</td>
<td>4 ml</td>
</tr>
</tbody>
</table>

### Non-terminal Blood Withdrawal

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximum Blood Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.1 ml per 10 grams body weight</td>
</tr>
<tr>
<td>Rat</td>
<td>1 ml per 100 grams body weight</td>
</tr>
<tr>
<td>Hamster/Gerbils</td>
<td>0.6 ml per 100 grams body weight</td>
</tr>
<tr>
<td>Rabbit</td>
<td>10 ml per kg</td>
</tr>
<tr>
<td>Nonhuman Primate</td>
<td>10 ml per kg</td>
</tr>
</tbody>
</table>

These stated volumes are the maximum blood to be withdrawn in a single bleed. At maximum, it can be repeated at two-week intervals. If more frequent bleeding is needed, the volume should be proportionally reduced. If more blood than this maximum volume is needed, the investigator must consult with the Veterinarian and receive IACUC approval.

### Physical Restraint

Physical restraint can cause both physical and psychological distress. According to the Guide for the Care and Use of Laboratory Animals (Eighth Edition), prolonged physical restraint should be avoided unless it is essential for achieving research objectives and is specifically approved by an Institutional Animal Care and Use Committee. If an animal will undergo prolonged restraint,
training of the animal to the restraint device should be conducted to assure acclimation of the animal to the restraint device. Restraint devices are not considered normal methods of housing. As such, the MSU IACUC has adopted the following guidelines regarding the use of physical restraint in animals.

Definition: The use of manual or mechanical means to limit some or all of an animal’s normal movement for the purpose of experimental manipulation.

Guidelines:
Restraint devices should be of suitable size, design, and operation to minimize discomfort or injury to an animal. The total time that an animal will have to be manually or mechanically restrained should be minimized. Methods for animal acclimation to the restraint device or activity must be defined. Animals that fail to adapt to the restraint device will be removed from the study. Animals must be observed at appropriate intervals, as approved by the IACUC. The veterinarian has the authority to temporarily or permanently remove any animal from the restraint procedures.

The following must be included in the animal care and use protocol:

- Justification of the use of physical restraint in the context of the research objectives.
- The duration of restraint, which should be the minimum required to accomplish the research objectives.
- A description of the method and duration of time needed for training or acclimation to the equipment and personnel. Positive reinforcement should be used whenever possible.
- A description of monitoring methods.
- A description of endpoints for temporary or permanent removal from restraint methods.

Polyclonal Antibody Production

The production of antibodies to specific and nonspecific antigens is a tool utilized in nearly all fields of biomedical research. Today antibodies are routinely made to a vast array of proteins, carbohydrates, fats, and nucleic acids. In addition modern biochemical, biosynthetic, and recombinant techniques have created increasingly pure antigens, both natural and synthetic, in an effort to induce very specific immune responses. Many of these newer antigens are small and generally weak immunogens and it is necessary to augment their immunogenicity with adjuvants. One of these antigens is Complete Freund’s Antigen (CFA). The Use of Complete Freund’s Adjuvant in rabbits should be utilized according to the following policy.

The Use of Complete Freund’s Adjuvant in Rabbits

Complete Freund’s Adjuvant (CFA) is widely used and considered to be the most effective adjuvant available for consistently producing high titer antibodies to diverse antigens. It is irreplaceable and vital for immunology research and antibody production at the present time. The Institutional Animal Care and Use Committee (IACUC) has developed the following guidelines to permit the continued use of CFA while maximizing antibody titer and minimizing the adverse effects of excessive inflammation, arthritis and other toxic effects.

CFA is a water-in-oil emulsion containing mycobacterial cell wall components that potentiate the humoral antibody response to injected immunogens. Adjuvant activity results from sustained release of antigen from the oily deposit and stimulation of a local immune response. The
improper or unnecessary use of CFA may cause inflammation, induration, and/or necrosis in laboratory animals.

The intention of the following guidelines is to minimize potential animal discomfort associated with the use of adjuvants in research. Unless specifically approved by the IACUC, antibody production using CFA at Montana State University must be done using these guidelines. Requests for deviations must be scientifically justified and will be considered on a case-by-case basis by the IACUC at the time of protocol review or in response to a request for protocol modification.

- Alternatives which reduce the number of animals (tissue cultures, chicken eggs, etc.) or utilize non-inflammatory adjuvants (Ribi, liposomes, incomplete Freund’s) must be considered.
- CFA should be used only when necessary, such as small amounts of available antigen and weak antigens. The USDA has declared that the use of CFA may cause more than momentary pain or distress and an alternative must be considered. A written narrative description of the methods and sources used to search for alternatives must be provided in the animal care and use protocol. Key words should include CFA and alternatives.
- CFA may be used only for the first (priming) dose. Subsequent immunizations should be with incomplete Freund’s or another adjuvant.
- The inoculums must be free of extraneous microbial or other particulate contamination. When possible, sterilization by filtration through a low binding 0.22 micron filter should be performed.
- Young adult rabbits (2.5-3.5 kg) should be used for primary immunization because of the vigorous antibody response. Immune function peaks at puberty and primary responses to new antigens decline with age.
- Immunizations involving the use of CFA will be performed by the ARC staff. Any exceptions must be approved by the IACUC and the Attending Veterinarian. If an exception is requested, the protocol must include a plan for appropriate training by the ARC Staff.
- Multiple injection sites must be separated from each other widely enough to insure continued blood supply. Anatomic sites which are weight bearing or used in restraint should be avoided (neck area in rabbits). It is strongly recommended to use sites close to the draining lymph nodes (ventral inguinal and axillary regions).
- Fur must be clipped and the site aseptically prepared with the appropriate antiseptics.
- Injections should be given subcutaneously. Any other route of administration requires additional scientific justification for IACUC approval.
- Injections must be divided into fractions so that no more than 0.1 ml is injected per site, with no more than a total volume of 1 ml (10 sites).
- Injection sites must be observed at least three times a week for four weeks after each injection. This should be properly documented in the animal record. If a lesion develops at any injection site, the Veterinarian must be notified so the animal can receive the appropriate treatment.

**Sanitation Frequency of Primary Enclosures for Rodents**

The Guide for the Care and Use of Laboratory Animals (p. 70-71) states, “By design, ventilated caging systems provide direct continuous exchange of air, compared to static caging systems that depend on passive ventilation form the macroenvironment. As noted above, decreased sanitation frequency may be justified if the microenvironment in the cages, under the conditions of use, is not compromised.”
The standard husbandry practice at the MSU Animal Resources Center is to sanitize rodent solid-bottom caging on a weekly basis. Under specific circumstances, sanitation may be performed every two weeks. Examples include rodents housed at a low density in individually ventilated cages, dams with new litters, and rodents housed in a high biocontainment environment. Noncontact items such as cage lids are sanitized monthly.

The MSU IACUC understands that sanitation of primary enclosures for rodents is typically performed on a weekly basis, but under circumstances such as those described above, may be extended to every two weeks. Animal Resources Staff, utilizing professional judgment, will be allowed to determine the frequency of rodent cage sanitation not to exceed once every two weeks.

**Single Housing of Rodents**

The *Guide for the Care and Use of Laboratory Animals* (p. 51) states, “An appropriate housing space or enclosure should also account for the animals’ social needs. Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility.” “Single housing of social species should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being.” (p. 64)

At Montana State University, every attempt will be made to socially house rodents. The IACUC recognizes that social housing is not always possible for experimental, health, and behavioral reasons. Some strains of rodents engage in barbering behavior and/or aggression which results in wounding of cage mates.

If individual housing is a required part of a research project, it should be discussed and justified within the animal care and use protocol. If single housing is required for health, behavioral, or breeding colony reasons it may be performed by the Animal Resources Staff using the appropriate professional judgment. Individual IACUC approval is not necessary for these cases.

**Single Housing of Large Animals (Livestock)**

This policy is applicable to Montana State University Department of Microbiology and Immunology (DMI). For the purpose of this document, livestock means horses, cattle, bison, sheep, and goats. The Johnson Family Livestock Facility (JFLF) is USDA accredited, but is not part of MSU’s AAALAC accreditation.

The default method of housing all livestock in the Johnson Family Livestock Facility (JFLF), the barn, the corrals, or the pasture is group or social housing, with at least 2 animals per group. Under the specific circumstances listed in this document an animal may be singly housed. This document is justification of any and all situations that may occur that would result in an animal, or a group of animals being housed in single pens.

If livestock are singly housed, it is for the health of the animal, as recommended by the consulting veterinarian, or as requested by specific PI’s approved protocol. During single housing, visual, auditory, olfactory, and protected tactile contact should be provided, where possible.
Quarantine of Animals
All livestock purchased by the IMID livestock unit, other than new born calves, are quarantined in groups with a minimum of 2 per group unless the consulting veterinarian recommends single housing as a result of severe aggression, a contagious disease, or other health reason (Category D, below).

All new born calves are quarantined inside single housing (Category A, below).

Single Housing Pens
Singly housed livestock, other than new born calves, will have visual, auditory, and olfactory access to other animals of the same species. They will be housed in a corral or in a 12x12 stall. Singly housed new born calves are housed in pens comprised of plastic dairy panels that have windows on each side and on the back. The front has two openings that allow the calf to put their head through and is where feed and water are freely accessed by each calf. The pens allow visual, auditory, olfactory, and protected tactile contact with other animals.

Justification for Single Housing
Livestock may need to be singly housed for a variety of reasons. The following are the categories of exceptions to group or social housing:

1. Category A- Quarantine of new born calves (and only calves) from birth up to a maximum of 8 weeks for the following reasons:
   i. Prevent spread of disease- Young calves require intensive care. Calves in quarantine often times will become ill, and keeping the calves in individual pens reduces the spread of illness and disease to the entire group. Since we have been using plastic panel pens the level of illness has dramatically decreased (calves cannot defecate on each other). Our individual animal records reflect this decrease in illness.
   ii. Reduce problems caused by calves suckling each other- Calves are prone to suck on each other, primarily on navel areas and ears. This can create health problems for the calf; specifically, it increases the amount of navel infections which can be fatal, sucking increases the size of umbilical hernias (a common problem in dairy calves), and sucking on ears can result in infected ear tags, ripped ears, and ear infections.
   iii. Increase ability to accurately monitor individual animals- Young calves and ill calves require strict monitoring of feed and water intake to ensure they are getting essential nutrients, calories and fluids. Calves must be taught to eat milk and texturized feed, and isolating the calves ensures that the amount of feed and fluid intake is accurate and adequate for each animal. When calves are sick, it is vital to know the consistency and frequency of their bowel movements; this is only possible by housing individually. Ill animals are less likely to compete with healthier animals for feed resulting in inadequate nutrition.

Note: Post quarantine procedure for new born calves- the majority of calves that have been in quarantine are moved outside into corrals and group housed until sold. If an animal is housed inside, either the JFLF or the barn, after the routine 8 weeks maximum quarantine time, the pen would be enlarged by removing a barrier wall allowing group or social housing. The number of animals housed in a group (minimum of 2) is dependent on animal weight and pen size. If an animal continues to be housed singly, it must meet the circumstances as stated in either category B, C or D, or it must meet one of the two requirements for being housed in true isolation as stated in this document.
2. Category B - Infectious disease experiments in livestock will follow housing requirements as outlined in Category A, above. Special husbandry and/or experimental techniques that are separate from the IMID large animal units SOPs as required by a PI must be in an IACUC-approved protocol.

3. Category C- Calves (and only calves) used for surgery. Normal behavior for calves includes suckling on each other, step on each other, or defecate on each other. This can result in sutures being broken, incision sites being opened or contaminated, or surgical procedures being compromised. Surgery calves will be single housed for a maximum of 3 weeks post operatively unless requested by the PI as stated in an IACUC-approved protocol.

4. Category D- As recommended by the consulting veterinarian for health reasons.

**Isolation**

Only two situations occur that require any animal to be truly isolated from other animals:

1. When any animal is critically sick and under the guidance of the veterinarian.
2. If a surgery or infectious disease study only requires one animal to be used at a time.

If truly isolated from other animals, additional enrichment is provided by increased human contact.

**Annual Review**

Dr. Bruce Sorensen and the livestock research manager, Kerri Jones, will review this document annually. If any modifications need to be made, this document will be modified and submitted to IACUC for review.

**Stabilization Period after Animal Transport**

All new animal arrivals, including mice, rats and other rodents, must undergo a minimum 48-hour stabilization period before experimental use. No component of the study may be initiated during this stabilization period. The stress associated with transportation has long been recognized to have profound physiological consequences. It is important to provide an adequate period before experimentation to allow animals to recover to a physiologically stable condition. This stabilization enhances both the validity of the experimental results and animal welfare.

**Tail Snips**

Rodents that are over 21 days of age must be anesthetized for a tail snip (amputation) procedure. Hemostasis must be ensured before the animal is returned to its cage.

**Animal Transportation**

a. Rodent transport on campus

When rodent transport is required from the ARC to another laboratory on campus, ARC approved cages with microisolator tops are required. Rodent transport on campus must be:

1) in a secondary container sturdy enough to be remained closed if dropped; or
2) on a cart during transport from the ARC to adjoining laboratory buildings.
For transport from ABSL2 areas, a secondary container is required and must be properly
discharged before leaving the area.

b. Rodent transport off campus to satellite facilities
If rodent transport off campus is required, a University-owned vehicle must be used. The use of a
personal vehicle is prohibited unless approved by the Attending Veterinarian prior to transport.
ARC approved cages with microisolator tops placed in a secondary container sturdy enough to
remain closed if dropped must be used during transport. Transport containers must be adequately
sanitized after each use.

c. Rodent transport to/from another regional research facility
On rare occasions rodents are transported to or from other regional research facilities using
private vehicles (e.g. McLaughlin Research Institute, University of Utah). Personnel must receive
approval from the Attending Veterinarian prior to transport.

d. Nonhuman primate transportation to Bozeman Deaconess Hospital
An ARC owned vehicle is required for transport of nonhuman primates to Bozeman Deaconess
Hospital. The procedure must be described in the animal care and use protocol and approved by
the IACUC. In addition, the Attending Veterinarian must approve prior to transport.

Unexpected Outcomes
The Guide for Care and Use of Laboratory Animals (8th Edition) states:

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

Examples of unexpected outcomes include, but are not limited to, the following:

a. An unexpected phenotype arising from a genetic modification or mutation that
   negatively affects animal well-being
b. Physical restraint that results in lesions, illness, or behavioral changes
c. A higher than expected morbidity or mortality rate.

It is the responsibility of the Investigator or their staff to promptly report to the IACUC any
unexpected outcome(s) that may negatively affect animal health or wellbeing.

All rodent breeding is centrally maintained by the ARC Staff and unexpected outcomes are
reported directly to the Attending Veterinarian (AV). The ARC Staff also performs nonhuman
primate daily health checks and any abnormality noted is promptly reported to the AV.
**Xenopus Frogs**

Amphibian oocytes and eggs are used for studies of molecular biology, embryology and biochemistry. Stage I-VI oocytes are often obtained by surgical laparotomy. While multiple major survival surgeries are discouraged as a rule, there might be certain circumstances where this is scientifically justifiable. One example is multiple survival surgeries for oocyte harvest in Xenopus frogs, due to marked variability in the quality of the oocytes from animal to animal and between experiments in a research project. In addition, the procedure is considered no more invasive than laparoscopic procedures, in that it requires a small incision directly over the area containing the oocytes which can then be collected without displacement or disturbing other visceral organs. Also, the procedure is rapid and the animals quickly return to normal feeding and activity.

The MSU Policy is as follows:

a. The number of laparotomies on frogs to obtain oocytes should be conditional upon the health of an individual frog, quality of the oocytes obtained, the age of the frog and probable duration of egg production.
b. A maximum of four recovery surgeries (with a final fifth terminal surgery) will be permitted, with a minimum period of six weeks between surgeries. Exceptions may be granted by the IACUC for compelling scientific reasons.
c. Investigators should alternate oocyte collection between right and left ovaries and rotate frogs so that the interval between surgeries is maximized.
d. Surgeries must be performed by trained persons using appropriate anesthesia, such as tricaine methane-sulfonate (MS-222). Cooling is not acceptable for use as an anesthetic.
e. Surgeries should be performed using aseptic technique appropriate for amphibians.

Single housing or small group housing for several days after surgery is ideal for a frog recovering from laparotomy. Frogs should be monitored during this period for appetite, activity and complications such as wound dehiscence or infection.

**Additional Resources**

A. Guide for the Care and Use of Laboratory Animals, 8th Edition (2010); page 117
C. IACUC Guideline on Egg and Oocyte Harvesting From Xenopus Laevis. Available at: http://www.upenn.edu/regulatoryaffairs/Pdf/10HarvestingOocytes.pdf

**Zebra Fish**

Public Health Service policy requires that all live vertebrate animals be included in 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.

In accordance with PHS policy, Montana State University IACUC sets forth the following policy regarding use of zebrafish in research:
a. Since they feel no pain or distress, zebrafish embryos less than 72 hours post fertilization may be used in research without IACUC approval. However, AVMA euthanasia guidelines must be followed.

b. The use of zebra fish 72 hours post fertilization requires IACUC approval.

c. Since early stages (up to 7 days post fertilization) feel no pain or distress, their numbers should be listed under Pain Category C and separately from zebrafish > 8 days post fertilization.

The investigator must determine the number of, and pain category for, zebrafish > 8 days post fertilization for IACUC approval according to the procedures described in the protocol.

Surgery and Anesthesia Policy

Anesthetic Cocktail Expiration

The expiration date is selected from the earliest expiration date of each component. Any adverse events must be reported to the Attending Veterinarian and a shorter date may be instituted at the discretion of the Attending Veterinarian.

Avertin (Tribromoethanol)

Avertin Usage

Avertin is the preferred anesthesia for most reproductive surgeries because: (i) unlike isoflurane, it is compatible with continual repositioning of the mouse during the procedure, for example under a dissecting microscope; and (ii) unlike ketamine/xylazine, the rate and duration of anesthesia are appropriate for most reproductive procedures, such as embryo transfers and ovary exchanges. Avertin should never be used on previously avertin exposed animals, due to induced inflammation and possible sensitization to subsequent exposure. Procedures using avertin should justify that:

1) Isoflurane/O₂ equipment will interfere with procedure, compromise survival, or compromise the mice.
2) That using a slower longer-lasting injectable anesthetic, such as ketamine/xylazine, will potentially compromise the procedure or increase risks to the mice.
3) Procedure can reliably be done in less than 15 min, including closure, application of analgesics, and return to recovery cage, such that animals remain anesthetized for duration of procedures.
4) No attempt will be made to re-dose for prolonged anesthesia.

Avertin Storage and Preparation

Avertin is not available as a drug substance, and must be prepared from approved-grade components. Avertin is prone to oxidation or other forms of chemical decay during prolonged or inappropriate storage, and therefore should be made fresh and stored as prescribed in the most recent release of “Manipulating the Mouse Embryo”, by RL Behringer et al, (Edition 4, January 2014), pages 197-198. Bottles must be labelled and dated. The solution must be checked prior to each use. If pH<5, the solution becomes discolored or if precipitate is present after shaking, these are indicators that the solution has decomposed and it should be discarded. A log of use is required and adverse events must be reported to the Attending Veterinarian and IACUC.
maximum length of storage is two months, but a shorter date may be instituted at the discretion of the Attending Veterinarian.

**Multiple Survival Surgeries**

Multiple survival surgical procedures can be justified if they are related components of a research project, if they will conserve scarce animal resources, or if they are needed for clinical reasons. It is unacceptable to subject an animal to multiple unrelated survival surgeries simply to save money or because the animals are in short supply.

**Pain Management**

Investigators are required to administer appropriate analgesics to all animals associated with a procedure that would normally require pain medication in humans. Questions concerning appropriate medication and dosage for a given species should be directed to Veterinarian. Exemption from this regulation is possible only on a protocol–by-protocol basis after Full IACUC review and approval.

**Rodent Surgical Guidelines**

**Pre-Operative**

1. Surgery should be conducted in a disinfected, uncluttered area, which promotes asepsis during surgery.
2. Prepare the animal by removing hair from the surgical site. Perform this procedure in an area separate from where the surgery is to be conducted.
3. Prepare the surgical site(s) with an appropriate skin disinfectant.
4. Surgeons should wash and dry their hands before aseptically donning sterile surgical gloves.

**Operative**

1. The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
2. Begin surgery with sterile instruments (autoclave, chemical sterilant, ethylene oxide) for each animal and handle them aseptically. Glass bead sterilizers may be used to sterilize the tips of instruments between animals or procedures.
3. Instruments and gloves may be used for a series of similar surgeries provided they are maintained clean and disinfected between animals.
4. Monitor and/or maintain the animal's vital signs.
5. Close surgical wounds using appropriate techniques and materials.

**Post-Operative**

1. Move the animal to a warm, dry area and monitor it during recovery. Return the animal to its routine housing only after it has fully recovered from anesthesia.
2. Provide analgesics as appropriate.
3. Generally, remove skin closures 10 to 14 days post-operatively.
4. Maintain a surgical record (e.g., surgery cage card) with procedure and date.
Survival Surgery

Regulations pertaining to survival surgery are very specific and include the following definitions:

- Survival Surgery includes all surgical procedures (even very minor ones such as vessel cut-downs) carried out on animals that are expected to recover from the anesthetic.
- Major Survival Surgery is defined as survival surgery in which a major body cavity (abdomen, thorax, cranial cavity) is entered or which has the potential for causing substantial impairment of physical or physiologic function.

All survival surgery must be carried out following aseptic technique, including the use of sterile gloves and properly sterilized instruments. This applies to all species including rats and mice. Major survival surgery on mammals other than rodents must be carried out in dedicated survival surgery facilities (ARC Room 111). Major survival surgery on rodents must be done according to the Guidelines for Survival Rodent Surgery.