



— RESEARCH INSTITUTE —
HEALTH & WELLNESS CENTER

**INSTITUTIONAL ANIMAL CARE AND USE
GUIDELINES**

Approved: 8/29/2024

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ADVERSE EVENTS, UNANTICIPATED OUTCOMES, ANIMAL WELFARE CONCERNS, AND NONCOMPLIANCE

Approved: 8/29/2024

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Revision History:

Purpose:

The Institutional Animal Care and Use Committee (IACUC) developed this guideline to promote transparency and consistency in its evaluation of animal welfare concerns, adverse events, unanticipated outcomes, and non-compliance events. In addition, it is intended to assist members of the ACHE community with determining what, when and how concerns regarding animal welfare should be reported.

Background:

ACHE is highly committed to ensuring that laboratory animals receive humane care and treatment while involved in research or training protocols in accordance with the ethical standards, laws, regulations, and policies/guidelines governing animal research. Safeguarding animal welfare is the responsibility of every individual involved with the care and use of laboratory animals.

The IACUC's charge requires review of each animal welfare concern in a timely and systematic manner and, when necessary, taking prompt and appropriate corrective actions.

Experimental use of animals in research, testing or teaching may occasionally result in serious, unanticipated, or adverse clinical outcomes. Adverse events, unanticipated outcomes, and noncompliance (including protocol deviations) must be reported to the IACUC to determine the cause and to prevent recurrence. Prompt reporting also helps the IACUC meet its federal requirement to monitor animal activities. Reporting is intended to be an interactive process and is not a unilateral cause for punitive action against investigators, but an effort to facilitate research effectiveness and improve animal care. The IACUC shall determine which events/outcomes must be reported to oversight agencies, whether protocol modifications are required (e.g., changes in procedures, monitoring, humane endpoints, etc.), or whether specific corrective actions are required to ensure animal well-being.

Definitions:

Adverse Events/Unanticipated Outcomes: An adverse event or unanticipated outcome is the occurrence of an unforeseen event that negatively impacts the welfare of research animal(s), involving pain, distress, and/or death of the animal. These are not identified as potential risks or outcomes in the approved IACUC protocol.

Examples of events that are required to be reported:

- i. Animal mortality or morbidity because of experimental conditions or outcomes not described in the approved IACUC protocol.
 - ii. Animal mortality or morbidity in excess of that described in the approved IACUC protocol.
 - iii. Animal mortality or morbidity in excess of humane endpoints described in the approved IACUC protocol.
 - iv. Unforeseen events that lead to the harm of the animal(s) or that cause obvious distress not justified and approved in the protocol, such as
 - 1. Unexpected phenotypes of genetically modified animals
 - 2. Protocol procedure complications.
 - 3. Unforeseen events that lead to the harm of the animal(s) or that cause obvious distress not associated with the approved protocol, including events associated with:
 - a. Animal housing and environmental conditions (e.g., mechanical, or electrical failures)
 - b. Animal husbandry and veterinary care (e.g., escape from primary containment, insufficient provision of food and/or water, non-response to veterinary care)
 - c. Hazardous material contamination (e.g., water or food supply contamination, spills/exposures, radiation leak)
 - d. Natural disasters
- b. Examples of events that are not required to be reported:
- i. Death or morbidity of animals described as expected in the approved IACUC protocol.
 - ii. Injury/illness unrelated to approved procedures and being treated by the clinical veterinarians
 - iii. Phenotypic abnormalities described in the approved protocol, common phenotypic abnormalities described in the literature (e.g., ulcerative dermatitis in specific strains), or phenotypic abnormalities that have no negative impact on animal welfare
- B. Non-Compliance: Can occur when an IACUC protocol, policies, procedures, or decisions are not followed. Examples of protocol noncompliance include:
- a. Conduct of animal-related activities without IACUC review and approval, or beyond the expiration date established by the IACUC
 - b. Failure to adhere to IACUC-approved protocols
 - c. Implementation of any significant change to IACUC approved protocols without prior IACUC approval

- d. Failure to correct deficiencies identified during the semiannual evaluation by the date set by the IACUC
 - f. Participation in animal-related activities by individuals not determined by the IACUC to be appropriately qualified and trained. (See IACUC Training Guidelines). Failure to monitor animals post-procedurally as necessary to ensure well-being (e.g., during recovery from anesthesia or during recuperation from invasive or debilitating procedures)
 - g. Failure to ensure death of animals after euthanasia procedures (e.g., failed euthanasia with CO₂)
- C. Animal Welfare Concerns: Concerns or deficiencies in the care and/or treatment of animals or any activities related to animal care that appear improper or inhumane should be reported.
- a. Examples of animal welfare concerns include:
 - 1. All conditions that jeopardize the health or well-being of animals
 - ii. failure of animal care and use personnel to carry out veterinary orders (e.g., treatments).

Reporting Adverse Events, Unanticipated Outcomes, Non-Compliance, and Animal Welfare

Concerns

Any member of the ACHE community may report adverse events, unanticipated outcomes, animal welfare concerns, and instances of suspected non-compliance with laws, rules, regulations, and policies/guidelines. Reports may be made to the Institutional Official (Office of the Vice President for Research), the IACUC Chair, the Attending Veterinarian, any member of the IACUC, the BRC facility manager, via an online form available on myACHE portal, or via the anonymous reporting hotline. ACHE prohibits unlawful retaliation against employees due to good faith actions in reporting wrongdoing allegations. Mechanisms for reporting concerns are posted in prominent locations in the animal facilities and on applicable ACHE websites with instructions on how to report concerns and to whom.

IACUC Actions Following a Report:

Conditions that could jeopardize the health or well-being of animals will be evaluated immediately.

The Attending Veterinarian (AV), Institutional Official (IO) and IACUC Chair are authorized to halt procedures that, in his/her judgment, do not comply with humane care and use of animals or conflict with institutional policy, until the IACUC can convene. Under Veterinary Authority, the AV (or his/her designee) may also euthanize animals deemed to be suffering with no apparent or impending relief.

Upon receipt of a reported concern, the IACUC Chair will add the matter to the agenda of the next scheduled IACUC meeting or convene a special meeting of the IACUC (this may also be in the form of an investigational subcommittee prior to the full committee meeting). After initial review of the complaint, the IACUC shall determine what actions are required.

If an activity is suspended, the IO shall promptly report that action to the appropriate federal oversight and accrediting bodies. The IO is required to promptly provide all oversight bodies including the Office of Laboratory Animal Welfare (OLAW), the National Science Foundation (NSF), the Department of Defense (DOD), U.S. Department of Agriculture (USDA) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) with a full explanation of the circumstances and actions taken with respect to:

- Any serious or continuing noncompliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals
- Any serious deviations from the provisions of the Guide Any suspension of an activity by the IACUC

The IACUC is obligated, through its Assurance with the Public Health Services' (PHS) Office for Laboratory Animal Welfare (OLAW), to self-report incidences of Noncompliance.

ANIMAL ACQUISITION

Approved: 8/29/2024

Last Reviewed: 8/29/2024

Revision History:

Purpose: The veterinary medical care of laboratory research animals is federally mandated. All animals must be acquired lawfully, and the receiving institution should make reasonable attempts to ensure that all transactions involving animal acquisitions are conducted in a lawful manner. (The Guide, 8th edition, p. 106.)

BRC staff is to be aware of the number and status of animals being received, to ensure the availability of space, to prepare for quarantine and assure biosecurity of animals, and to address special housing conditions, etc., as applicable.

Acquisition of animals is permitted after the appropriate IACUC protocol has been approved, when sufficient animal numbers are available, and when the vendor/supplier and/or health status of the animals has been approved.

Animal numbers are updated and tracked constantly for reports required by federal and accreditation agencies and for internal and external audits.

Acquisition of all animals:

1. Animals that are to be housed in the core animal facilities require BRC approval and must be coordinated through the BRC when coming from commercial vendors, currently approved protocols, or other institutions.

2. Transfer request forms are required when animals are transferred from one protocol to another or from one location to another within the BRC.

3. Process:

When acquiring animals from an approved vendor or other outside institutions, the process is initiated by completing the Animal Acquisition Form on the BRC site under myACHE.

For transfer of animals from a different location on campus (a different room), a different protocol, or a different per diem account number, the transfer must be reviewed and approved by the BRC prior to occurring. The process may be initiated by submitting an Animal Transfer Request form found on the BRC site under myACHE.

4. **Acclimation: All animals should be allowed at least four days to acclimate to their new environment prior to being entered into a study.**

ANIMAL IDENTIFICATION AND GENOTYPING

Approved: 8/29/24

Last Reviewed: 9/10/24

Revision History: 9/10/24-mandatory analgesia for toe clipping

Animal Identification:

Recommended methods of identification include implantation of microchips, indelible skin markers, ear tags, ear notch/punch/snip, and tattooing.

Genotyping:

The preferred methods of genotyping are via ear notch/snip or buccal swab

Collection of tissues for genotyping is a common procedure when using genetically modified mice. Researchers should remove the least amount of tissue necessary to perform genotyping and consider using genotyping techniques that do not permanently alter the animal (ex: collection of hair, fecal pellets, buccal swabs) when scientifically appropriate. The use of rusted or dull equipment is unacceptable. Scissors and reusable punches should be sharpened or replaced at appropriate intervals based on use. Blades should be discarded after each session.

Tail Snip: A common method for gathering tissue is tail snipping. Refer to these steps when performing tail snipping:

1. Restrain or anesthetize the rodent (must be anesthetized if over 17 days of age).
2. Starting with sanitized scissors, cut the defined length of distal tail. (no more than 2mm)
3. Monitor the animals to ensure hemostasis after the rodents are returned to the cage. If needed, apply digital pressure, or “Kwik Stop” to control bleeding.
4. Take great care to remove all tissue from the scissors or scalpel after each animal. Disinfect scissors/ scalpel with 70% ethanol between animals to reduce risk of infection and prevent sample contamination.
5. Take no more than 1-2 mm (about 0.08 in) of tissue. Other analytical and confirmatory techniques may require more tissue, however, the amount taken may not exceed 2 mm (about 0.08 in). If additional samples are needed from the same animal, the PI must first secure IACUC approval of the request in the protocol and include justification for why additional tail sample must be collected and why alternative sample collection sites cannot be used. Analgesic administration may also be required depending on the request.

Ear Notching: If ear notching mice for identification, the piece of tissue punched out should be used for genotyping. This process does not require anesthesia.

Ear Snip: A small portion (2-3 mm) of the edge of the pinna is cut off with sharp scissors to obtain tissue. This can be done on mice once the ears have developed (> 8 days of age) and does not require anesthesia.

Toe Clip: Toe clipping is not a standard procedure and must be justified in the IACUC protocol.

The IACUC (Institutional Animal Care and Use Committee) regards toe clipping as a potentially painful procedure and it should be categorized as USDA (United States Department of Agriculture) pain category D (analgesia must be provided). Toe-clipping is allowed only for altricial rodents pre-weaning and toes may only be removed from rodents after the toes are no longer webbed (usually between postnatal days 4 and 5) and only up to seven days of age. Every reasonable effort should be made to minimize pain or distress, including limiting the number of digits clipped to one digit per rodent. If possible, it is preferable to remove toes from a hind paw rather than a forepaw, especially if the animals will be used in studies that include grip strength testing. **Toe clipping should only be used when no other individual identification method is feasible.** Toe clipping can be performed on conscious neonate mice or rats to identify individual animals. Whenever possible, removed toe tissue should be used as part of the genotype analysis technique thus reducing the amount of additional tissue that would need to be collected. The use of toe clipping must be scientifically justified in the IACUC protocol.

Toe Clipping Procedure:

Procedure:

1. Mouse or rat should be held in gloved hands.
2. Toes to be clipped should be cleaned with 70% alcohol.
3. One toe may be removed from three feet only.
 - a. Do not remove more than one toe per foot, and do not cut the small most medial/inner toe (hallux/dewclaw).
4. Very sharp scissors with fine pointed tips or scalpel blades must be used for this procedure. They must be sterile or disinfected with accelerated hydrogen peroxide (AHP) disinfectant for at least 5 minutes before use.
 - a. The instrument must be cleaned with alcohol after each animal. Scissors must be re-sterilized or disinfected with AHP after every 5 animals. Scalpels must be discarded after 5 animals.
5. Bleeding should be immediately controlled by gentle fingertip pressure.
6. Pups should be quickly returned to their dams. Cages containing toe clipped neonates should be checked 5 minutes after toe removal to confirm complete hemostasis.

This procedure can be done without anesthesia. **Exceptions to the maximum age of animal will not be granted.** This procedure should be implemented with caution in animals used for behavioral research or research that involves the analysis of mobility (e.g., Rotarod, treadmill, etc.). Studies have demonstrated decreased grip strength in toe-clipped animals depending on the age the toes were removed.

Hair: Tufts of hair (n=2 tufts per mouse, > 20 follicles) are plucked from the animal using tweezers or hemostats to obtain samples. Samples can be collected at the neckline between the shoulder blades. Animals should not have exposed patches of skin following sampling, as only small tufts are needed. This method does not require anesthesia. Care should be taken to avoid contamination with fomites and with hair from cage mates of the animal to be assessed.

Fecal Pellets: Samples of feces (n=3 pellets) can be collected directly from the animal at the time of defecation, or from the cage floor of individually housed animals within 24 hours of defecation. Epithelial cells shed in the feces are the target tissue type for processing and analysis. This method does not require anesthesia.

Buccal Swabs/Saliva: Salivary samples to harvest epithelial cells from the mouth can be performed on rodents once they are a few days old; this method does not require anesthesia. Individual sterile mini-cotton swabs (rubbed against both inner cheeks per swab) should be used to sample cells. Care should be taken within the mouths of animals to ensure gentle swabbing.

CONFIDENTIALITY AND CONFLICT OF INTEREST

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose: To address matters of confidentiality and conflicts of interest.

Definitions:

- **Confidential Business Information** — Commercial or financial information considered to be confidential because disclosure may: (1) Impair the Government's ability to obtain necessary information in the future; or (2) Cause substantial harm to the competitive position of the individual or business entity who provides the information.
- **Proprietary Information** — Information or data belonging to an owner or proprietor, who may have exclusive rights to the manufacture and sale of a specific item.
- **Trade Secret** — Any formula, pattern, device, or information that is used in business which provides a competitive advantage.
- **Sensitive Information** — Information or data in which disclosure, loss, misuse, alteration, or destruction may adversely affect national security or other government (usually Federal) interest.

Procedures:

- Committee members are required to comply with a signed agreement which provides this University's principles on confidentiality (Non-Disclosure Agreement (NDA)) - exhibited below
- IACUC members receive training on confidentiality and conflict of interest as part of IACUC Orientation

Conflict of Interest Concerns:

If an applicant submitting a protocol believes that an IACUC member has a potential conflict, the applicant may request that the member be excluded from the review of the protocol. The request must be in writing and addressed to the IACUC Chairperson at least five working days prior to the protocol's distribution to the committee for review. The request must contain evidence that substantiates the claim that a conflict of interest exists with the IACUC member(s) in question. The committee may elect to investigate the applicant's claim of the potential conflict. The Chair will determine whether there is a need to assemble a subcommittee of at least two (2) members to evaluate whether the potential for conflict exists. The Chair or IO will be the deciding vote in situations where an agreement cannot be reached. The Chair will notify the applicant and the committee member in writing of the subcommittee's determination. If it is

determined that the potential for a conflict of interest exists, the IACUC member to which the concern is directed will be excused from the committee discussion and voting. The application will not be distributed to the committee members. If it is determined that the potential for a conflict of interest does not exist, the application will be distributed to the member in question, and he/she/they will be allowed to participate and contribute to the committee discussion on the protocol and vote.

Appeals may be brought to the attention of the Institutional Official. If an applicant or any other employee believes that a member of the IACUC has engaged in action or actions which has or have placed the committee member in conflict of interest, that complainant may bring allegations of misconduct to the attention of the Institutional Official for further investigation.

Exhibit NDA:



**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NON-DISCLOSURE AGREEMENT**

SCOPE

The "Undersigned," as a member of the Institutional Animal Care and Use Committee (IACUC) has been asked and appointed to assess research and teaching/training conducted at Arkansas Colleges of Health Science, hereafter referred to as "ACHE", and ensures that research and teaching/training is conducted in an ethical manner, with the highest standard of care according to applied federal, state, local regulations, and institutional policies and guidelines.

Adequately evaluating research and teaching/training programs requires an exchange of information. It is understood; therefore, that the Undersigned may be exposed, either verbally, visually, or in writing, to material, data, discoveries, ideas and other information, which ACHE considers Proprietary, Confidential, or Privileged.

CONFIDENTIALITY

This Agreement thus encompasses any information deemed Confidential or Proprietary provided to the Undersigned in conjunction with duties as a member of the committee. As such, the Undersigned agrees to hold all Confidential or Proprietary trade secrets ("information") in trust or confidence and agrees that it shall be used only for contemplated purposes and shall not be used for any other purpose or disclosed to any third party. Written confidential information provided for reviews shall not be copied or retained. All confidential information (and any copies and notes thereof) shall remain the sole property of ACHE.

Furthermore, the Undersigned shall not make any use of such information in their own research, teaching/training or commercial development activities without written consent from the Vice-President of Research. It is understood that this nondisclosure obligation shall not apply to any information known by the Undersigned or generally known in the field or industry prior to the date of this agreement, or becomes, through no fault of the Undersigned, common knowledge within the field or industry. The Undersigned agrees not to disclose or utilize, directly or indirectly, any Confidential or Proprietary information belonging to a third party in fulfilling this agreement. Furthermore, the Undersigned confirms that their performance of this agreement is consistent with ACHE policies and any contractual obligations they may have to third parties. If the Undersigned is an ACHE employee, the Undersigned further agrees and acknowledges that entering into this Agreement shall have no effect over the employee's responsibility to report allegations of discrimination or harassment, including their obligations to report consistent with ACHE's Sexual Harassment and Sexual Misconduct Policy.

Lastly, the Undersigned shall not discuss, communicate, or disclose any information that is considered official committee business to third parties without written consent from the Vice-President of Research.

By signing below, I acknowledge and agree to abide by the terms of this agreement.

Resources:

The Guide for the Care and Use of Laboratory Animals 8th edition p. 26

Animal Welfare Act and Regulations p. 69

ACHE Policy Manual Section 1.5 - Conflict of Interest Policy

ACHE Non-Disclosure Agreement

CONTROLLED SUBSTANCES

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Use of Controlled Substances

Definitions:

- **Registrant:** A registered person with the DEA and/or Arkansas Board of Pharmacy to use Controlled Substances.
- **Registration:** The permit obtained from the DEA and/or the Arkansas Board of Pharmacy to use Controlled Substances.
- **Research:** Any scientific investigation.

Overview

This document establishes the guidelines for the authorization, procurement, storage, control, and disposal of all Controlled Drugs for animal research purposes.

The document will be periodically reviewed to ensure compliance with existing laws and regulations. If there is a conflict between these guidelines and applicable laws and regulations, the more restrictive will govern.

Drugs that are Controlled Substances are listed in the following laws/regulations:

- i. Schedules I to V of Title 21 of the Code of Federal Regulations (CFR) Section 1308.

Scheduled drugs are listed at <http://www.deadiversion.usdoj.gov/schedules/index.html>

Registrants should receive training on the management of Controlled Drugs. The forms associated with these guidelines can be found on the BRC (Biomedical Resource Center) website. Registrants should provide registration information to the BRC.

Applicability

This document applies to the acquisition, use, storage, disposal, or related activities, including record-keeping, that involve Controlled Substances used in animal research conducted by ACHE (Arkansas Colleges of Health Education) researchers or that take place in ACHE facilities.

This Policy does not cover Controlled Substances used by a physician, pharmacist, dentist, podiatrist, veterinarian, or any other healthcare practitioner in providing health/veterinary care for their patients or clients.

Summary of Responsibility under this Policy:

Anyone who uses Controlled Substances is responsible for knowing and following all applicable federal, state, and local laws/regulations and ACHE policies. Supervisors must ensure that anyone reporting to them follows these laws/regulations and will be held accountable for violations.

Procedures

Training

Training regarding Controlled Substances used in Animal Research is provided by the BRC as part of research compliance.

Registration

All persons at ACHE who want to conduct Research that includes using Controlled Substances must register with the appropriate agencies. Registration requirements differ depending on whether the researcher is a Practitioner and the type of Research to be conducted (e.g., DEA and the Arkansas Pharmacy Board have specific requirements before the approval of controlled substances for use in research).

Information on permit/registration processes can be found on the BRC site under myACHE.

Security and Storage

Researchers are responsible for ensuring that the Controlled Substances used in their research are kept secure to prevent theft, loss, unauthorized access, or removal.

The Registrant cannot hire or utilize any employee or agent whose work requires them to have access to Controlled Substances if that person has been convicted of a felony relating to Controlled Substances or has had a DEA Registration denied, revoked, or surrendered for cause.

The Registrant cannot transfer or provide Controlled Substances to any other Registrants for use in those persons' research. The Office of Research will review requests for transfers of Controlled Substances on a case-by-case basis provided there are substantial reasons, and the appropriate documentation with DEA and the Arkansas Board of Pharmacy are in place before the transfer.

Reporting Loss or Diversion of Controlled Substances

Employees, students, and other agents must report any suspected loss or theft of Controlled Substances to the Registrant. The Registrant must promptly report any theft or significant loss as follows:

Controlled Substances

- **DEA:** Within one business day of discovery, complete [DEA Form 106](#) (For Controlled Substances only)
- **Arkansas Board of Pharmacy**

Inspections

By law, an inspection of registered sites may be conducted by agents of the DEA or other authorized licensing, police, law enforcement agencies, or ACHE entities. The Registrant should promptly notify the Office of Research of any inspection or pending inspection and provide the office of research with a copy of any inspection report received.

Ordering and Procurement

Researchers must order and purchase their own controlled drugs for use in animal research. The drugs must be ordered directly by the Registrant.

If a Controlled Substance in a registrant's possession will be shipped outside ACHE, the Registrant should contact the Office of Research

Disposal

The researcher is responsible for properly disposing of Controlled Substances when the substances expire, the DEA Registration is not renewed, or the Registrant no longer conducts Research using Controlled Drugs, or the researcher leaves ACHE.

ACHE requires that the Registrant utilize any Controlled Substances ordered under their Registration solely for their Research as described by DEA.

Information on disposal can be found on the BRC site under myACHE.

Records

Researchers must keep accurate records on the receipt, storage, use, and disposal of Controlled Substances. Records must be maintained for 3 years after the final disposition of Controlled Substances. An official inventory must be performed every two years. The DEA will look for the following documentation:

- Log of persons with access to the room where Controlled Substances are stored
- Log of persons authorized to use Controlled Substances
- Log of all orders and receipts of Controlled Substances
- Initial and biennial inventory log
- Running use and disposition log for each container of Controlled Substances

EUTHANASIA

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History

Purpose

This document establishes the standards for euthanasia of laboratory animals at ACHE and has been created to ensure that euthanasia complies with the requirements of the Animal Welfare Act and Regulations, the Guide for the Care and Use of Laboratory Animals, 8th Edition (the Guide), and the AVMA Guidelines on Euthanasia of Animals (AVMA Guidelines).

Regulatory or Accreditation Authority

9 C.F.R. §2.31 – Institutional Animal Care and Use Committee

(xi) Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, §1.1 of this subchapter, unless a deviation is justified for scientific reasons, in writing, by the investigator.

*Guide for the Care and Use of Laboratory Animals, 8th Edition, November 2013.
Euthanasia. pp. 123-124*

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, ISBN 978-1-882691-54-8

Scope

Applies to all animals euthanized at ACHE

Definitions

Euthanasia: the humane disposition of an animal accomplished by a method that minimizes or eliminates pain and distress. This is routinely accomplished by rapid unconsciousness and subsequent death.

Procedures

A. General

To minimize animal suffering, laboratory animals must be euthanized either as described in the protocol at established endpoints, or expeditiously if criteria for humane endpoints have been reached. Animals must be continually observed and never be left unattended during the euthanasia procedure. All methods used must result in the confirmed death of the animal; for many methods this requires a secondary physical method after the primary chemical method to ensure death. Animal carcasses and tissues must be properly disposed of after euthanasia in the appropriate BRC freezers.

B. Authority

If BRC animal facility staff discover animals suffering from unrelieved pain or distress, they will attempt to contact the PI or designated alternate. If the PI or alternate cannot be reached, euthanasia may be performed at the discretion of the BRC veterinarian.

C. Protocol Requirements

The method of euthanasia must be consistent with the AVMA Guidelines, appropriate for the species and age of the animal, and performed by trained personnel. Protocols must include a description of methods used for euthanasia, including method(s) to confirm death.

D. Rodent Euthanasia

- 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 (e.g., decapitation, exsanguination, cervical dislocation, radical bilateral thoracotomy, tissue perfusion, or dissecting of a vital organ). The animal must be completely non-responsive to noxious stimuli (confirmed by lack of response to hind foot pad pinch on each foot) before any physical method is performed. All agents used must be pharmaceutical grade, unless [non-pharmaceutical grade agents](#) are scientifically justified and approved in the IACUC protocol.
- The techniques listed below are methods **commonly approved** in protocols for the euthanasia of rodents. **Other methods outlined in the AVMA Guidelines on Euthanasia are acceptable when approved in the IACUC protocol.**
- Precocial rodents are to be euthanized as adults.

Chemical Methods

1. **Carbon Dioxide Inhalation/administration (≥ 7 days of age):** Purified 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 30-70% of the chamber or cage volume/minute, allowing CO₂ to enter the chamber and induce unconsciousness 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, see 'Temporary Holding Cages' below.

To ensure compliance with the *AVMA Guidelines*, a precision CO₂ gauge/regulator with a pressure reducing valve or flow meter must be used (unless it is an automated pre-calibrated with these components integrated into the system e.g., EZ Ssystems). Units in the BRC necropsy area are set to the appropriate flow rate. Units in decentralized care must be similarly equipped or a flow meter may be used set to the proper flow rate for the cage size used. Inspection and verification that flow rate complies with the AVMA Guidelines should be confirmed triennially by the BRC or lab staff.

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 from all sides, be a size that permits full posture to be expressed and be disinfected between uses to remove the potential distress caused by exposure to remaining pheromones. CO₂ is denser than room air and will remain at the bottom of the chamber. Do not place animals in a pre-charged (containing CO₂ from the previous group) chamber.

2. **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**. Sodium pentobarbital containing 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. For rats, it is recommended to refine the method by combining sodium pentobarbital with Lidocaine in the same syringe to reduce the irritant effects of sodium pentobarbital – please consult the BRC veterinarian for guidance.
3. **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 approved in the IACUC protocol and adequately scavenged to prevent personnel exposure and animals must not contact the liquid anesthesia. Animals may need to be exposed for prolonged time periods to ensure death.
4. **Euthanasia while under Anesthesia:** When animals are fully anesthetized as at the end of a non-survival surgery, methods such as bilateral thoracotomy, exsanguination, removal of a vital organ 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 if the following conditions are met:

- Up to 10 compatible adult (> P21) mice may be placed in a temporary holding cage for up to 30 minutes and holding cages are never left unattended.
- The holding cage has adequate floor space for the age, size, and number of animals
- If fighting is observed, mice must be immediately separated.
- Adult males \geq 6 weeks old from different cages should never be combined.
- For pups >12 days:
 - An established breeding cage can be euthanized as a single cage regardless of the number of animals (e.g., approved trio breeding cage with 12 pups >12 days of age)
 - When combining cages, no more than 10 animals (including adults and pups >12 days of age) can be combined.
- For pups \leq 12 days of age):

- Cages with any pups \leq 12 days of age cannot be combined with pups $>$ 12 days of age or non-parental adult mice.
- No more than 2-4 litters of pups \leq 12 days of age can be placed in single layer within the cage without adult mice, and ALL pups must be \leq 12 days of age.

Physical Methods

As secondary methods: Chemical methods must be followed by a confirmatory physical method. Decapitation, exsanguination, cervical dislocation, bilateral thoracotomy, tissue perfusion, or dissecting of a vital organ must occur after the animal has been determined to be non-responsive to noxious stimuli.

As primary methods: Physical-only methods of euthanasia such as decapitation or cervical dislocation of un-anesthetized animals must be approved by the IACUC with appropriate scientific justification in the IACUC protocol. The PI must ensure that personnel are experienced or properly trained, and documentation of competence is required.

Species-specific requirements:

1. **Mice and Gerbils:** Decapitation, cervical dislocation
2. **Rats and Hamsters:** Decapitation, cervical dislocation (animals must be less than 21 days and/or weighing less than 200 grams).
3. **Guinea pigs:** Decapitation only. 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. Precocial (able to independently feed and move almost immediately at birth) young should be euthanized as adults (for example: guinea pigs). Altricial neonates are defined as less than postnatal day 12.

Anesthetic overdose, as listed in the chemical methods above, can be used.

Inhalation anesthetics:

1. Exposure time for inhaled anesthetics may take up to 50 minutes for euthanasia to be successful. Adequate exposure must be provided and followed by cervical dislocation, decapitation, or bilateral thoracotomy
2. CO₂ (Prior to Physical Method): Neonatal rodents (up to 10 days) are resistant to the effects of CO₂ and up to 50 minutes can be required to achieve death. CO₂ may be used for narcosis of neonatal rodents provided it is followed by another method of euthanasia (e.g., decapitation) Neonates should not be combined into cages with non-parental adult mice. Place no more than 2 - 4 litters of neonates in a single layer in a mouse or rat cage. Place the cage within the euthanasia chamber and fill with CO₂ for at least 4 or 5 minutes with CO₂ tank regulator set to displace 30-70% of the cage volume per minute. Neonates can then be removed, and physical method performed.

Decapitation using scissors or sharp blades is acceptable as a sole means of euthanasia.

Hypothermia followed by secondary physical method can be used for animals ≤ 7 days of age. Fetuses and altricial neonates can be gradually cooled until anesthetized, careful to prevent direct contact with ice or precooled surfaces, followed by decapitation, bilateral thoracotomy, or removal of major organ(s)

Rapid freezing in liquid N₂ is acceptable for less than 5 days of age

Non-Rodent Mammal Euthanasia

Use of an anesthetic agent for euthanasia must be at an [overdose](#), not an anesthetic dose.

To confirm death, the administration of any chemical agent used for euthanasia must be followed by a physical method from which the animal cannot recover, such as bilateral thoracotomy or fixative perfusion. The animal must be completely non-responsive to noxious stimuli before any physical method is performed. All agents used must be pharmaceutical grade.

The techniques listed below are methods commonly approved by the IACUC for the euthanasia of non-rodent mammals. Appropriate restraint for the species must always be applied. Sedation, anesthesia, or tranquilization may be necessary for some species or individual animals prior to the administration of the euthanasia agent(s). **Injectable anesthetic overdose:** Intravenous injection of an anesthetic agent may be an acceptable method; however, intracardiac injections are acceptable only when the animal is adequately anesthetized. Intraperitoneal injection may be approved for smaller species (mice, rats, birds). Sodium pentobarbital containing agents are recommended, though other injectable anesthetics may be acceptable.

Euthanasia while under anesthesia: When animals are fully anesthetized (e.g., at the end of non-survival surgery), methods such as bilateral thoracotomy, exsanguination, perfusion, or intravenous or intracardiac injection of potassium chloride are acceptable.

Maintenance and Use of Decapitation Equipment:

Equipment used for euthanasia of unanesthetized 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 and rinse with 70% ethanol to promote drying.

Blades that are in use should be sharpened at least annually and blades that have been out of service must be sharpened before the first procedure. The guillotine apparatus should be lubricated periodically with silicone.

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 sharpened by a professional sharpening service. **Sharpening and maintenance records need to be available during IACUC inspections and upon request.**

Lab staff must be appropriately experienced or trained (see Physical Methods above). When using decapitation equipment always make sure hands and fingers are clear of blade path. The use of plastic restraint cones (i.e. [Decapicones®](#)) is recommended to restrain adult animals as it may reduce distress from handling, minimize the chance of injury to personnel and improves

positioning of the animal in the guillotine.

Zebrafish Euthanasia

Approved methods for zebrafish euthanasia by age and agent:

Adults 7 days post fertilization (dpf) and older include:

- Tricaine (MS-222): Immerse fish in a solution of tricaine methane sulfonate (Finquel or Tricaine-S). The solution must be buffered with sodium bicarbonate to a pH of 7.0-7.5. Stock preparation is 4g/L buffered to pH7 in sodium bicarbonate (at 2:1 bicarb to MS- 222). Euthanasia dosage 300ug/ml or 7.5ml stock solution to a total of 100ml. Fish must remain in the solution for 30 minutes following cessation of opercular (gill) movement.
- Rapid chilling: Submerge fish in 2 -4⁰ C chilled system water (5 parts ice to 1 part water). Confirm with a thermometer that the water is ≤ 4°C before placing the fish. They should never be in direct contact with ice. Adult fish should be exposed for 10 minutes following cessation of opercular movement (small bodied (≤3.8 cm) tropical and subtropical stenothermic finfish only)

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.

Embryos 0-3 days post fertilization:

- Tricaine or rapid chilling may be used as above, but embryos < 3 dpf should be followed with an adjunctive method such as dilute bleach. Add dilute bleach solution (1 part sodium hypochlorite 6.15% to 5 parts deionized water) to the water for 5 minutes to ensure embryonic lethality.
- After one of the above methods has been performed, acceptable adjunctive physical methods for all stages of development will be done including (for non-transgenic animals) maceration (use of a specially designed mechanical apparatus having rotating blades or projections, causing immediate fragmentation) or placement of the animal carcasses in the freezer. Carcasses will be transferred to biohazard bags at -20oC for at least 24 hours and then disposed of as potentially hazardous tissue by the BRC.

Disposal

All carcasses are placed into red biohazard bags. The bags must be sealed and stored in the appropriate BRC freezers until removed by the animal waste management contractors.

Training

Only trained individuals may perform euthanasia. Training can be provided by scheduling with BRC personnel.

Note: Compressed CO₂ in cylinders is the only AVMA recommended source of CO₂ for euthanasia purposes.

References:

- American Veterinary Medical Association. 2020. AVMA Guidelines for the Euthanasia of Animals, 2020 edition.
 - Koerber AS and Kalishman J. 2009. Preparing for a Semiannual IACUC Inspection of a Satellite Zebrafish (*Danio rerio*) Facility. Journal of the American Association for Laboratory Animal Science. **48**: 65-75.
- NIH. 2020. Guidelines for Use of Zebrafish in the NIH Intramural Research Program

EXPIRED DRUGS AND MEDICAL MATERIALS

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Background

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

Responsibility

Each PI and laboratory staff are responsible and accountable for ensuring that expired materials are not used for animal research and that expired drugs, medical supplies and/or devices are properly disposed of by their expiration date. Proper labeling will allow the IACUC and researchers to distinguish expired from nonexpired materials stored in secondary containment.

Expired drugs are not to be used for survival or non-survival procedures. Expired medical materials used for non-survival procedures must be labeled as such.

Definitions

Drug: for this policy, any regulatory agency approved or investigational substance, agent, biologic, or chemical listed in a pharmacopeia, chemical supply catalogue, synthesized or isolated in a laboratory, and administered to animals by any route, including injection, inhalation, topical application for use in the investigation, diagnosis, cure, mitigation, treatment or prevention of disease or biology in humans or animals. Examples include antibiotics, controlled substances, anesthesia agents, analgesia agents, etc.

Controlled Substance: Any drug, substance or analog compound that falls under any of the five (I-V) Schedules identified by the United States Department of Justice/Drug Enforcement Administration and as defined by the Controlled Substance Act (CSA).

Medical material: A non-bioactive substance intended to be administered to animals for use in the diagnosis, cure, mitigation, treatment, or prevention of disease, or a substance intended to affect the structure or function of the body. (e.g., catheters, sutures) Food is not considered a medical material.

Expiration Dates & Proper Labeling

All chemicals used on or in animals must have an expiration date clearly labeled on the container. The expiration date is the date printed on the label/package for materials with a manufacturer's expiration.

Expired materials found in the vivarium may be discarded at any time by the Biomedical Resource Center Staff or the IACUC unless materials are clearly labeled “not for use in animals” and are stored separately from materials for use in animals.

1. **Secondary containers that hold an unadulterated solution** (*i.e.*, a drug or material from an original stock to which no drug has been added) must be clearly labeled with the name of the drug or material and the expiration date of the original stock.
2. **For dilutions, preparations, reconstitutions, or mixtures of drugs or fluids** made by the PI or their laboratory staff, prepared using aseptic, and under proper storage conditions:
 - a. **Best practice** – is to open or prepare the smallest quantity of drug required for that day’s work and discard any remaining drug at the end of the day. Containers must be labeled with name, final drug concentration or dilution, and the new expiration date as soon as they are prepared. Expiration dates of each component in a drug mixture should be listed in laboratory records so that the potential expiration date of the mixture can be accurately verified upon request (*i.e.*, during a facility inspection).
 - i. A pharmaceutical grade drug with an added diluent or substance (*e.g.*, combining Ketamine and Xylazine) used as an analgesic, anesthetic, antibiotic, or euthanasia agent **expires 30 days** beyond the date of preparation, or until the manufacturer expiration date if sooner than 30 days.
 - ii. Sterile fluids used as diluents expire on which ever date occurs first:
 1. The manufacturer expiration date,
 2. 72 hours after opening when administered intravenously,
 3. Or 30 days after opening when administered via a route other than intravenous.

When PIs wish to access sterile diluents multiple times (*i.e.*, to obtain small volumes for administration and drug mixing), the investigators can do so only if they do not add any chemical to the fluid, they access the fluid(s) aseptically and they store the fluid(s) as recommended by the manufacturer.

- b. **Acceptable practice** – If a drug is diluted or mixed with another compatible drug and put into a sealed, sterile container, the container must be labeled by name, final drug concentration or dilution, preparation date, and new expiration date.
3. **For powdered forms of drugs or compounds that do not list an expiration date** - the PI should determine the stability of the drug to identify a reasonable shelf-life. This is commonly obtained from the manufacturer. If stability is unknown, best practice dictates that the drug should not be used beyond one year. Use a light-proof, airtight container and label the container with a one-year expiration and the name of the drug.
4. **For drugs or solutions that are reconstituted for use:** Reconstituted drugs and compounds that do not contain expiration or efficacy guidelines in the directions must be labeled for expiration **30 days after reconstitution** in a glass container. The final container must contain the name of the drug and/or compound with the expiration date.

5. **For Chemicals** - PI-made chemical preparations in secondary containment must be labeled by name, hazards present, chemical concentration, preparation date, and new expiration date. Chemical transferred or aliquoted to secondary containment must be labeled by name, hazards present, and expiration date of the original stock. Expired chemicals must be labeled as “EXPIRED,” prior to disposal.

For questions regarding chemical labeling and disposal please contact the laboratory supervisor and/or the appropriate ACHE committee (IBC/Safety)

6. **Medical materials:** The use of expired medical materials for survival procedures is not permitted. All medical materials and supplies used in live animals, including acute procedures, should be individually labeled with an expiration date or a sterilized date. Medical Materials such as catheters, surgical instruments, sterile gloves, suture materials are often labeled with an expiration date to assure sterility. In general, after the expiration date, these materials must be discarded.

Expired materials capable of being re-sterilized should be labeled with a new expiration date appropriate for the package being used. If the PI obtains information from the manufacturer that provides documentation that a particular material or instrument has been shown to remain sterile beyond the date on the packaging, a request to use the item in survival studies will be considered by the IACUC.

Instruments or supplies sterilized by the investigator must be marked with an expiration date based on the date of sterilization. They must be marked with an external “process indicator” (e.g., autoclave tape) and must contain a sterilization indicator (usually a chemical indicator strip that indicates exposure to sufficient heat or ethylene oxide to sterilize). Cloth-wrapped or paper-wrapped sterilized equipment has a shelf life of six months. Plastic-wrapped sterilized items (e.g., peel-packs) have a shelf life of six months. Medical materials and devices without a manufacturer-provided expiration date must be labeled by name, and PI- determined, lab-specific expiration date.

- a. Expired sterilized items capable of re-sterilization must be re-sterilized and labeled with the new expiration date.
 - b. Items sterilized in-house must be labeled with the date of sterilization and an expiration date.
7. **Discarding expired drugs, chemicals, and medical materials:**
 - a. No expired drugs or fluids may be administered to animals for any research or instructional purpose, including terminal procedures.
 - b. Expired drugs, solutions, dilutions, etc., must be labeled as “EXPIRED - not for use in animals,” prior to disposal. Items must be clearly labeled and segregated from non-expired items until they can be appropriately discarded. Do not store it in animal use areas.

IACUC Recommendations:

1. Appropriately discard all expired surgical materials and supplies.

2. Prepare and access all medications aseptically and store according to manufacturer guidelines.
3. It is best practice to open or prepare the smallest quantity of drug required for that day's work and discard any remaining drug at the end of the day.
4. Uncontaminated multi-dose vials must be stored according to manufacturer's recommendations and can be used up to the manufacturer's expiration date if they show no signs of contamination. Use a new, sterile needle for each entry into the container. Use should be discontinued if the rubber stopper appears damaged, solution is discolored or there are other signs of contamination.
5. Expired anesthetics, analgesics, and euthanasia solutions must never be used, and must be disposed of properly.
6. Perform regular checks of your inventory and properly dispose of drugs or medical materials that will expire in the forthcoming month. Please contact the BRC, Safety Committee, IBC, or IACUC for assistance with disposal.
7. Store all expired drugs and medical materials in a clearly labeled ("Expired – Do Not Use/Expired – Not for Animal Use") container or drawer while they await pickup for disposal or return to manufacturer.
8. Follow all federal requirements for DEA registration, storage, and disposal of controlled substances.

FOOD AND FLUID RESTRICTION

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose

This guideline establishes standards and expectations for researchers performing food and/or fluid regulation in healthy animals for experimental purposes. This policy does not apply to animals restricted at the advice of the veterinary staff or in preparation for anesthesia.

Note: both regulation and restriction will need to be included in the IACUC protocol under the food/fluid restriction section.

What is food **Regulation**? Food regulation is the scheduled access to food or fluid sources during which an animal may consume as much as desired at regular intervals.

What is food **Restriction**? Food restriction is the provision of rations such that the volume of food or fluid is strictly monitored and controlled.

Food

Animals should be fed palatable, uncontaminated diets that meet their nutritional and behavioral needs daily, or according to their requirements, unless the protocol they are being used in requires otherwise (Guide page 65).

Water

Animals should have access to potable, uncontaminated drinking water according to their requirement (Guide page 67).

General Guidance

1. Food or fluid regulation or restriction may be required for some physiological, neuroscience, and behavioral research protocols. The type and extent of regulation or restrictions must be described and justified in the approved IACUC protocol that covers the animal(s). Researchers must state in their protocol the necessary level of regulation, potential adverse consequences of regulation or restriction, and methods for assessing the health and well-being of the animals. The least restrictive schedule that will achieve scientific objectives while maintaining animal well-being should be utilized (*Guide* 2011 pg. 30-31).
2. Written records must be maintained for each animal to document daily food and fluid consumption. Daily health checks must be performed by the PI (Principal Investigators) to monitor the hydration status, and behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol (*Guide* 2011 pg. 31).

3. Investigators utilizing food or water regulation, or restriction protocols **must communicate with the Biomedical Resource Center staff**, by means of a special cage card, about periods of restriction and about rest periods when full or supplemental water/food can be provided.

Water Restriction – General Information

1. Because of individual variation in water requirements, use of average guidelines for water intake or urine output may not be appropriate but will be evaluated by the IACUC when submitting the protocol.
2. Water restricted animals must be monitored daily for health as indicated by stability in weight, stability of performance in the experimental protocol, development of signs of dehydration (skin turgor, mucous membrane dryness, urine output and specific gravity, blood analysis), and development of signs of stress. Body weight must be monitored daily during the first week of restriction and at least weekly thereafter.
3. Any use of water restriction must be scientifically justified and approved by the IACUC.
4. Disturbances of normal activities can signal stress, including changes in normal sleep cycles, abnormal social interactions, and emergence of abnormal behaviors such as stereotypic behaviors, cage chewing, hair picking, abnormal vocalizations, and aggression. Careful evaluation of the animal for physiologic signs of dehydration should be performed should signs of stress become evident.

Food Restriction – General Information

1. Due to variation in food requirements and nutritive status, use of average guidelines for food intake may not be appropriate. Mature or obese animals can tolerate greater food restriction than younger/thinner cohorts.
2. Food restricted animals must be weighed at least weekly for health as indicated by stability in weight. An animal should not experience any more than a 15% loss of body weight unless it is scientifically justified. Other parameters for measuring health appropriate to the species should be monitored in consultation with the veterinarian. PI staff must record weight and observations on the provided BRC cage cards.
3. Any use of food restriction must be scientifically justified and approved by the IACUC.
4. Full access to food must be provided to pregnant dams at least 2 days prior to parturition to prevent cannibalization of the pups at birth.

Food or Water Restriction Procedures

The information provided in this section describes the responsibility of each person involved in the management of water or food restriction for rodents and the procedure for taking animals on and off ad libitum food or water.

1. Water restriction period is the days during which the animal's water consumption will decrease. Food restriction period is the days during which the animal's food consumption will decrease.
2. Individual variation in food and water consumption based on factors such as strain, sex, age, health status must be considered when establishing food or fluid regulation or restriction paradigms.
3. Food or water restriction is prohibited for seven days post-surgery, to ensure the animal's physiological parameters return to normal before restrictions are introduced.
4. Water restriction is prohibited for animals receiving antibiotics as damage to the kidneys may occur.
5. Exceptions to these guidelines must be scientifically justified and approved by the IACUC.

Responsibility of the PI and/or Lab Members

Before starting the restriction period:

1. Record the weight of each animal in lab records and on the cage card.
2. Place a "Feed by PI" or "Water by PI" cage card on the cage. These are provided by the BRC. Indicate that the research staff will provide food or water depending on the desired type of restriction.
3. Unless the approved and planned experiment involves total withdrawal of food and/or water, provide the animal with at least 2/3 of its average daily food and/or water consumption.

Daily

1. Confirm that the animal has received its daily food or water ration by initiating the record one day at a time on the room work chart.

Ongoing Monitoring:

1. Records on body weight must be maintained and made available to the BRC staff, veterinarian, and/or to the IACUC upon request. If the animal's body weight reaches the humane endpoint criteria established in the IACUC approved protocol the animal must be euthanized. The established guideline is no more than 15% weight loss as an endpoint.

Important criteria that the IACUC uses for review:

1. The least restriction that will achieve the scientific objective should be used.
2. Criteria must be defined (such as weight loss or state of hydration) for temporary or permanent removal of an animal from the experimental protocol.

3. A monitoring program should be established and should include records of the following: ensuring that food and fluid intake meets nutritional needs, daily observation of the animals (including weekends and holidays), weight records, and any clinical changes that are to be used as criteria for temporary or permanent removal for a protocol. The BRC will provide an area on the work chart records for the PI or their staff to complete this task. Work charts are on all animal room doors. Work charts will be stored as records by the BRC for at least three years. The BRC also provides cage cards used to record animals' and water bottle weights for restrictions.

GENETICALLY MODIFIED ANIMALS

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Background: 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

Procedures:

1. The first offspring of a newly generated genetically modified animal line should be carefully observed from birth into early adulthood for signs of disease, pain, or distress. Investigators may find that the phenotype precludes breeding of genotypes or that unexpected infertility occurs, which could lead to increases in the numbers of animals used and revision of the animal use protocol.
2. When the initial characterization of a genetically modified animal reveals a condition that negatively affects animal well-being, this should be immediately reported to the IACUC (Institutional Animal Care and Use Committee), and more extensive analysis may be required to better define the phenotype. Such monitoring and reporting may help to determine whether proactive measures can circumvent or alleviate the impact of genetic modification on the animal's wellbeing and to establish humane endpoints specific to the genetically modified animal line.

HEALTH RECORDS

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History

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Background Information

1. The Animal Welfare Act, *Guide for the Care and Use of Laboratory Animals* and the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* require that appropriate experimental, breeding, and health records are maintained for animals used or produced for teaching or research.
2. Daily observations must be made of all animals to assess health and well-being. Personnel conducting observations need to be trained to recognize physical and behavioral health concerns.

Record Requirements for all Animals

1. A daily record/entry of animal well-being checks. This can be done via a room care sheet to indicate the animals have been evaluated or via individual records for USDA covered species.
2. Documentation of experimental or teaching procedures performed, including drugs administered (dose, route, and frequency), samples collected, observation for clinical response, surgical description with anesthesia and post-operative care records and method of euthanasia or final disposition. Group records may be kept for non-USDA covered rodents and fish.
3. Records of observations, veterinary care, treatment, and experimental or teaching procedures must be structured and readily accessible to the attending veterinarian or designee, as well as inspectors and site visitors from regulatory and oversight bodies (e.g., IACUC, OLAW, USDA, AAALAC).

Documentation Required for Animals Showing Signs of Illness, Injury, or Other Departure from Normal Health Status Includes:

1. Identification of the animal (s) or group(s)
2. Pertinent history
3. Clinical information such as results of physical examination, the behavior of the animal, and notations regarding observed abnormalities, illnesses, and/or injuries

4. Records of diagnostic tests and interpretation including necropsy findings if necropsy is indicated.
5. Tentative / provisional diagnosis
6. Diagnostic and treatment plan
7. Record of veterinary care/treatment/interventions, including documentation of the drug, dose, route, and frequency of administration for medications and non-pharmaceutical treatments such as special diets, wound care, or physical therapy.
8. Record of the animals' clinical response and follow-up. This constitutes regular assessment of the animal's condition during the treatment/observation period. Documentation of clinical response is in addition to the routine daily health check provided for all animals.
9. Resolution of the problem (e.g., diagnosis, treatment, return to a normal state, euthanasia).
10. In some cases, a composite record may be used for a group of animals for preventative health treatments, experimental or teaching procedures, or post-operative monitoring. At minimum, such records should include: a list of the animal numbers/IDs, notation that each animal has been checked, documentation of any abnormal findings or occurrences, and a description of any therapeutics given including drugs, doses, and routes of administration.
11. Written entries must be signed or initialed by the author.
12. There should be documentation that veterinary oversight and authority is in place regarding veterinary care. For example, if there is a veterinary phone consultation, the record should indicate the veterinarian's identity, date of consultation and any relevant information related to the consultation.

Location of Records

For animals maintained in a vivarium, treatment record(s) must be maintained in a manner that allows for immediate access (e.g., in or near the room where the animals are housed). This is especially important for post-operative animals or animals with anticipated abnormal observations. Easily accessible records allow ready communication between the research, veterinary and animal care staff.

Additional Information

1. This does not require a standard form for animal care and medical records. Whatever the form, the record(s) should be structured, readily available, legible and should contain all pertinent clinical information (as discussed in section C above) to justify the tentative diagnosis and any treatment provided. The final resolution for each case must also be properly documented.

2. Health Cases may be brought to the attention of any BRC staff member who will then consult the veterinarian. Communication can be in person, via email, or via the online platform.
3. Additional information and examples of record templates are available from the BRC staff.

Resources

[USDA Animal Welfare Act](#)

[Guide for the Care and Use of Laboratory Animals](#), 8th ed. 2011. National Research Council.

HUMAN ENDPOINTS

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

(Adapted from NIH Office of Animal Care and Use Guidelines)

Introduction:

Endpoints are a critical component of every protocol which must be reviewed by the Institutional Animal Care and Use Committee (IACUC). It is ideal when the scientific aims and the study objectives can be accomplished without adverse effects, pain, or distress to the animal. However, this is not always possible and careful consideration must be given to the:

- Scientific requirements of the study.
- Expected and/or possible adverse effects that the research animals may experience (pain, distress, illness, etc.)
- Probable time course and progression of those adverse effects.
- Earliest most predictive indicators of present or impending adverse effects.

Where pain or distress is a necessary part of the study, a humane endpoint must be used and approved by the IACUC. A humane endpoint is the earliest scientifically justified point at which pain or distress in an experimental animal can be prevented, terminated, or relieved, while meeting the scientific aims and objectives of the study. Such endpoints are preferable to death or moribundity since they minimize pain and distress and may be considered refinements.

The effective implementation of endpoints requires properly trained and qualified individuals to perform both general and study-specific observations on research animals at appropriate time points. The assessment criteria and required response from the PI or designee must be clearly defined within the protocol, and the use of study-specific animal assessment records should be considered. Studies must be designed to minimize pain and/or distress.

When initiating a new set of experiments, the potential for pain and/or distress may be unknown and/or the nature and extent of resulting morbidity and/or moribundity and/or mortality cannot be anticipated. Therefore, smaller pilot studies may be useful as they can be instrumental in the development of an appropriate endpoint and determining animal numbers.

Finally, investigators performing studies that include pain, or distress should reassess the necessity for any morbidity, moribundity, or mortality throughout the studies and refine the endpoints whenever possible. Post-approval monitoring is another tool the IACUC can use to determine if endpoints are being used as intended and if further refinements are possible.

Morbidity:

Protocols that include morbidity as an endpoint or that include animal procedures that have the potential to cause adverse sequelae should address the following as they relate to the expected outcomes:

1. Criteria to identify the humane endpoint:
 - a. There are several examples in the literature that might be considered for most species, including:
 - i. Evaluation of five aspects of an animal's condition as described by Morton and Griffiths⁶. These are: body weight, physical appearance, measurable clinical signs, unprovoked behavior, and response to external stimuli.
 - ii. Clinical observations used in cancer research and toxicological studies include changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces, and locomotion¹² (Table 1).
 - iii. Objective endpoints such as weight loss or body condition (BC) scoring can be applied to most species. Body condition scoring may be useful in younger animals that are actively growing or oncology studies where tumor growth increases weight concurrent with catabolism.^{2,3, 14}
 - iv. The clinical signs, depending on severity, duration, and response to appropriate therapy, that *may* constitute an endpoint in most species include, but are not limited to:
 - Rapid or progressive weight loss. Young or growing animals should have weight assessed by using growth charts typical for their species/strain, body condition scoring or comparison to untreated age & sex matched conspecifics.
 - Anorexia (lack or loss of appetite) or failure to drink
 - Debilitating diarrhea
 - Dehydration/reduced skin turgor or edema
 - Sizable abdominal enlargement or ascites
 - Dermatitis or other conditions not responsive to treatment
 - Rough hair coat/unkept appearance (Hunched posture)
 - Lethargy or persistent recumbency
 - Loss of righting reflex or failure to maintain equilibrium
 - Coughing, labored breathing, nasal discharge, gasping
 - Jaundice, cyanosis, and/or pallor/anemia

- Neurological signs (seizures, paralysis, paresis, circling/head tilt, blindness)
- Bleeding from any orifice
- Self-induced trauma with exposure of underlying muscle
- Any condition interfering with daily activities (e.g., eating or drinking, nest- building, ambulation, elimination, or normal postural movements)
- Excessive or prolonged hyperthermia or hypothermia
- Additional signs in neoplasia studies that may constitute an endpoint include, but are not limited to:
 - A tumor burden greater than 10% body weight. In an adult mouse, a tumor should not exceed 20 mm (about 0.79 in) in any one dimension; in an adult rat, a tumor should not exceed 40 mm (about 1.57 in) in any one dimension. Formulas for calculating tumor size can be found in the literature¹⁸⁻²⁰⁻²⁴.
 - Tumors that ulcerate and/or become necrotic and/or infected.
 - Tumors that interfere with eating or impair ambulation.

Table 1- Examples of Clinical Observation Based Endpoints. Montgomery, C.A. Jr. (1990), *Cancer Bulletin* 42:230-237

Parameter	Observations
General Appearance	Dehydration, decreased body weight, missing anatomy, abnormal posture, hypothermia, fractured appendage, swelling, tissue masses, prolapse, paraphimosis
Skin and fur	Discoloration, urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur
Eyes	Exophthalmos, microphthalmia, ptosis, reddened eye, lacrimation, discharge, opacity
Nose, Mouth, and Head	Head tilted, nasal discharge, malocclusion, salivation
Respiration	Sneezing, dyspnea, tachypnea, rales
Urine	Discoloration, blood in urine, polyuria, anuria
Feces	Discoloration, blood in the feces, softness/diarrhea
Locomotor	Hyperactivity, coma, ataxia, circling, muscle, tremors,

Considerations for aging studies:

1. Senescent animals may naturally exhibit several clinical signs that would indicate significant morbidity in younger animals. Aging animals may also experience certain benign ailments at an increased incidence. Genotype, background strain, chronological age, and sex should therefore be considered in the process of developing endpoints in these studies.
2. For lifespan studies, where clinical signs of morbidity associated with aging are expected and necessary for the scientific aims and objectives of the study, the endpoint of the study should be as objectively described as possible by the investigators. The literature suggests that in aging rodents there are changes in serial measurements of temperature, and body weight that correlate with imminent death^{12,15,17,18}. However, these changes may not be predicative at the individual animal level. Where more subjective endpoints such as deterioration in general health or quality of life are used, the assessment will rely on a veterinarian's observation and judgment in consultation with the Principal Investigator as to when the endpoint has been reached. Additional parameters to consider in evaluation of larger species could include complete blood counts, blood chemistries, urinalysis, and other minimally invasive techniques to evaluate organ function.

Unexpected/spontaneous non-experimentally related conditions:

Conditions may arise in breeders or other "normal" animals or during the conduct of research that are unexpected and unrelated to the research being conducted. Conditions may be specific, such as a spontaneous tumor, or more of a general deterioration of health/quality of life. These conditions can still have a significant impact on animal welfare and experimental results and must be addressed appropriately. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water must be euthanized. In less severe cases that may include pain or distress, the unexpected/unrelated condition should be assessed for the impact on animal welfare and experimental results. If the condition impacts experimental results, the animal should be euthanized. If it does not affect the experimental results (this would also be the case with a breeder or a normal untreated animal), standard veterinary treatment must be provided. If standard veterinary treatment would affect the experimental results, the animals should be euthanized unless withholding treatment for the condition(s) is specifically approved in the protocol. If the condition worsens following treatment, the animal should be euthanized, dependent on veterinary judgment and with Principal Investigator consultation. In some animal models, such as specific phenotypes in Genetically Modified Animals (GMAs), conditions that impact animal welfare can be expected to continue or reoccur. If not already present, these should be included in a modification to the protocol as "expected resultant affects" to alert animal care staff and specify appropriate additional animal care and/or euthanasia

Monitoring for endpoints:

- A plan for monitoring the animals both before and after a change in any of the above aspects, providing care if appropriate, and increasing the level of monitoring as necessary, should be in place. Monitoring or clinical care on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal

care and veterinary staff. Unless otherwise specified in the protocol, monitoring and taking appropriate actions is the investigative staff's responsibility, including on weekends and holidays.

- Identify the personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia. Checklists or score sheets may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented.

Death or Moribundity:

Death or Moribundity as an endpoint is strongly discouraged. While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there are studies that may require moribundity or mortality as an endpoint. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. Commonly used signs of moribundity include, but are not limited to:

- lack of responsiveness to manual stimulation.
- immobility; and/or
- an inability to eat or drink.

In these studies, animals are permitted to die or become moribund, because of experimental procedures. In some cases, pain relieving measures are not used because such measures may compromise the experimental integrity of the study. Examples of research proposals that may have death or moribundity as an endpoint include infectious disease studies, drug and toxicity studies, and cancer research. The following guidelines are suggested to assist the IACUC in reviewing proposals with death or moribundity as endpoints. Note that death as an endpoint is only allowed in rodent studies.

Required Information for Protocols Utilizing Death or Moribundity as an Endpoint:

1. The scientific rationale for death or moribundity as an endpoint, including:
 - a. What alternatives were considered, why morbidity as an endpoint cannot be used instead of death, and how alternatives will be used whenever possible.
 - b. Why measures to relieve pain and/or distress cannot be utilized.
 - c. The number of animals that will be allowed to reach moribundity/death and justification for it being the minimum necessary to achieve the scientific objectives.
 - d. Whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.
2. A plan for the following animal care and monitoring procedures:
 - a. Animals involved in experiments that may lead to moribundity or death will be monitored at least daily by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for at least the following: abnormal posture, rough hair coat, head tucked into abdomen, exudates around eyes and/ or nose, skin lesions; abnormal breathing, difficulty with ambulation, decreased food or water intake, lack of response to stimulation, or self-mutilation.

- b. The frequency of observation will be increased when animals exhibit the above described or other signs of morbidity. Monitoring on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff. A system should be in place where designated personnel, including a veterinarian, are notified when animals show signs of disease. An assessment of the animals' condition should be made as soon as possible, and a plan of action established.
- c. Consideration will be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely. Deceased animals must be promptly removed.
- d. Written records will be kept of monitoring frequency and observations.

Imaging/Biomarkers:

The use of serial imaging or biomarkers may permit the detection of experimental endpoints that precede the development of significant clinical signs. Consideration should be given to their use especially in studies that could result in morbidity, moribundity, or mortality.

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16. Yorke A, Kane AE, Hancock Friesen CL, Howlett SE, O'Blenes S. [Development of a Rat Clinical Frailty Index.](#) *J Gerontol A Biol Sci Med Sci.* 2017 Jul 1;72(7):897-903.
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References - Tumor Size:

18. Bullard DE, Schold SC Jr, Bigner SH, Bigner DD. 1981. [Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell lines.](#) *J Neuropath Exp Neurol* 40:410- 427.
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NON-PHARMACEUTICAL GRADE SUBSTANCES

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

A **pharmaceutical grade compound** is any active or inactive drug, biologic, reagent, etc. which is approved by the FDA or for which a chemical purity standard has been written/established by any recognized pharmacopeia (book/compendia) such as: US Pharmacopeia (USP), National Formulary, British Pharmacopeia.

1. For all species, pharmaceutical grade compounds should be used whenever they are available.
2. For all species, permission must be obtained from the IACUC to administer any non-pharmaceutical chemical compounds. Cost is not considered an adequate reason to employ non-pharmaceutical chemical compounds.
3. For all species, any non-pharmaceutical grade substances may be acceptable when:
 - a. There are no equivalent pharmaceutical grade compounds available
 - b. Approved by the IACUC
 - c. Scientifically necessary
 - d. There is a schedule of monitoring that allows the detection of adverse events related to the use of non-pharmaceutical grade compounds
 - e. Issues related to quality assurance such as proper preparation, storage, and shelf life* have been addressed in the IACUC protocol form. These factors would include:
 - i) Appropriate drug reconstitution, preparation, and/or compounding
 - ii) Drug purity, sterility, pH, osmolality, concentration, etc.
 - iii) Drug safety, efficacy, and shelf-life
 - iv) Site and route of administration
 - v) Training, experience, and performance of personnel involved in usage
 - vi) Personnel Responsible for monitoring preparation and use Side effects and adverse reactions

Note: Not all factors may be applicable.

*If the “shelf life” is not obtainable, it is recommended that the drug solution be re-prepared each day it is used.

Regulations: USDA states the following regarding use of non- pharmaceutical grade compounds in animal experimentation:

“Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures. Non-Pharmaceutical-grade chemical compounds should only be used in regulated animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical- grade product. Cost savings alone are not an adequate justification for using non- pharmaceutical-grade compounds in regulated animals.”

OLAW: May investigators use non-pharmaceutical-grade compounds in animals?

OLAW agrees with the USDA that pharmaceutical-grade¹ chemicals and other substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and / or interfere with the interpretation of research results. However, it is frequently necessary to use investigational compounds, veterinarian or pharmacy-compounded² drugs, and / or Schedule I³ controlled substances to meet scientific and research goals.

Guide for the Care and Use of Laboratory Animals; Eighth edition:

“The use of pharmaceutical-grade chemicals and other substances ensures that toxic and unwanted side effects are not introduced into studies conducted with experimental animals. They should therefore be used, when available, for all animal-related procedures. The use of non- pharmaceutical –grade chemicals or substances should be described and justified in the animal use protocol and be approved by the IACUC; for example, the use of non – pharmaceutical-grade chemicals of substance may be necessary to meet the scientific goals of a project when a veterinary or human pharmaceutical-grade product is unavailable.” pp. 31.

A pharmaceutical grade compound is a drug, biologic, or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary (USP-NF), or British Pharmacopeia (BP).

Veterinary compounding is the customized manipulation of an approved drug by a veterinarian, or by a pharmacist upon the prescription of a veterinarian, to meet the needs of a research study.

United States Department of Justice Drug Enforcement Agency controlled substances Schedule I and II-IV drugs may be used in biomedical research according to the standards of the Code of Federal Regulations 1301.13.

PERSONNEL TRAINING

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose: Delineate required training for the IACUC (Institutional Animal Care and Use Committee), investigators who are listed on an IACUC approved protocol and/or personnel involved in the regular animal care or treatment of vertebrate animals and cephalopods covered under a protocol.

Rationale: The Animal Welfare Regulations (AWR) in Section 2.32 require the institution to ensure that all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use are qualified and trained to perform their duties. Additionally, the institution must make training and instruction available in the specific areas outlined under 2.32(c).

Definitions: CITI: Collaborative Institutional Training Initiative, the online training platform which supplies training content

The IACUC requires that certain training must be completed before investigators can work with or provide care to vertebrate animals and cephalopods. This policy also includes personnel involved in regular animal care or treatment of vertebrate animals covered under an IACUC-approved protocol and BRC (Biomedical Resource Center) staff.

IACUC Members:

Each IACUC member will be provided with a copy of the following:

1. The PHS (Public Health Service) Policy for the Humane Care and Use of Laboratory Animals.
2. The National Research Council (NRC): The Guide for the Care and Use of Laboratory Animals.
3. The ARENA/OLAW IACUC Guidebook.
4. The AVMA Guidelines on Euthanasia.
5. A copy of the OLAW (Office of Laboratory Animal Welfare) approved Assurance.

All new IACUC members are provided an orientation that covers the functions of an IACUC as well as an overview of the Animal Care and Use Program which provides training on methods for reporting concerns, humane practices of animal care and use (reduction, refinement, and replacement and methods to minimize pain and distress), use of hazardous agents when working with animals, zoonosis hazards, and what to do in the case of an injury. New members are mentored in protocol review and semi-annual review activities until they are comfortable conducting these activities on their own. In addition, members are required to take the “Essentials for IACUC Members” module offered through www.CITIProgram.org. The Chair and community members are also encouraged to complete CITI courses related to their roles.

IACUC members are kept apprised of new policies and procedures via an IACUC dedicated SharePoint site.

Completion of one continuing education course is required annually and ongoing training on assorted topics is provided at a minimum of once a year during a designated IACUC meeting. Continued enrollment in the occupational health program is required.

Scientists, Animal Technicians, and other Personnel involved with Animal Care:

(See Chart Below)

Training must be completed prior to working with animals and prior to protocol approval. All personnel are required to attend an orientation which provides training on methods for reporting concerns, humane practices of animal care and use, reduction, refinement, and replacement, as well as methods to minimize pain and distress, use of hazardous agents when working with animals, zoonosis hazards, and what to do in the case of an injury. A facility orientation is also required during which rodent users receive hands on training for animal handling and restraint. All personnel must be continually enrolled in the occupational health program.

All personnel and supervisors with roles in the care and use of animals at the Institution are to take online CITI training relevant to the species they will be working with as well as the CITI course entitled "Working with the IACUC." Completion of one continuing education course is required annually. The course must be related to animal welfare or reduction, refinement, and replacement. This can be completed through webinars, CITI courses, AALAS (American Association for Laboratory Animal Science) courses, seminars, conferences, etc. The IACUC has delegated the determination of appropriate applicability of annual continuing education courses to the BRC staff.

BRC personnel must complete the above training and be trained on all BRC standard operating procedures, specific to the responsibilities within the animal care and use program. They are also trained to recognize common laboratory animal ailments.

Personnel performing surgery must complete a training session with the veterinarian on proper use of anesthetics, analgesics, and tranquilizers for the species studied as well as aseptic technique and suture training as applicable to the project.

Training in experimental methods such as specific animal manipulations and techniques will be conducted on an as needed basis for the types of research being conducted and the species being studied.

Note: For investigators transferring from other facilities at which they have received similar training, verification of previous training may be accepted in lieu of some Institutional required training. Acceptance of previous training in lieu of the Institution's training is solely at the IACUC's discretion.

	Orientation	Occupational Health Enrollment	Facility tour and hands on training	Complete CITI modules	Training with BRC to recognize common diseases
Personnel Working with Mice	X	X	X	X "Working with the IACUC" "Working with Mice in Research Settings"	
Personnel Working with Rats	X	X	X	X "Working with the IACUC" "Working with Rats in Research Settings"	
Personnel working with Fish	X	X	X	X "Working with the IACUC" "Working with Fish in Research Settings" OR "Working with Zebrafish in Research Settings"	
Personnel working with Amphibians	X	X	X	X "Working with the IACUC" "Working with Amphibians in Research Settings"	
BRC personnel	X	X	X	X "Working with the IACUC" And all pertinent species modules	X

PILOT STUDIES

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

ACHE (Arkansas Colleges of Health Education) is committed to observing Federal policies and regulations and the AAALAC Inc standards for the humane care and use of animals. This document provides guidance on pilot studies.

This document covers all animals on ACHE premises used for research, teaching, training, breeding, and related activities, hereinafter referred to collectively as “activities,” and is applicable to all persons responsible for conducting activities involving live vertebrate animals at or under the auspices of ACHE.

The Guide for the Care and Use of Laboratory Animals states that when novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC (Institutional Animal Care and Use Committee), and veterinarian. A system of communication with the IACUC should be in place both during and after such studies (Guide p.28).

ACHE follows the *Guide* pilot study requirements.

Guidelines

It is acceptable to ask for animals that will be used in pilot experiments. Experiments can be used to perfect technique, demonstrate feasibility, or provide a justification for proceeding with larger, more tightly controlled experiments. Another justification for the use of pilot studies could be to estimate variability. The value so obtained can then be used to calculate group sizes in upcoming experiments by power analysis.

Responsibilities

It is the responsibility of the investigator to:

1. Propose a pilot study, as deemed necessary.
2. Submit IACUC protocols
3. Make modifications to protocols to secure IACUC approval.
4. Ensure adherence to approved IACUC protocols.
5. Ensure humane care and use of animals is accomplished.
6. Communicate to the IACUC the results of any pilot study.

It is the responsibility of the IACUC to:

1. Assure that the number of animals to be used in an animal use protocol is appropriate.
2. Review and discuss reports submitted by the investigator pertaining to their pilot study.

Procedures

For any new techniques, or when endpoints are proposed, an investigator may need to show that it can work on a small number of animals (pilot study) before requesting a larger number of animals.

An IACUC protocol must be completed and submitted for IACUC review and approval.

A pilot study may be requested by the IACUC during protocol review.

For studies involving new procedures:

1. If little is known about a specific procedure, the IACUC may approve and oversee a pilot study designed to assess both the effect of the procedure on the animals and the skills of the research team.
2. The investigator must report to the IACUC, in writing, the findings of the pilot study.
3. If the study will continue as a larger study, the protocol that contains the pilot study must be amended to include the larger study. The amended protocol will not be approved by the IACUC until the results of the pilot study are known and communicated to the IACUC.

For alternative endpoints:

1. The use of a pilot study is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC, and the veterinarian.
2. For these studies, a report must be made, in writing, to the IACUC during and after the study. Based on the report, the IACUC will determine if the alternative endpoint can be used on a larger scale.

Reporting Requirements

Pilot Studies – for protocols which will consist of a pilot study or series of pilot studies, as well as protocols which propose a pilot study as part of its total experimentation, typically only one report will be due to the IACUC. Information about reporting requirements and timelines will be noted on the initial protocol approval certification letter or from the IACUC Coordinator. Your report would briefly cover the following:

1. The total number of animals used to date.
2. A summary of experimental results/outcomes thus far.
3. A summary of animal health outcomes, particularly any unexpected or adverse outcomes compromising animal health/well-being beyond what was anticipated.
4. The PIs own interpretation of any results/outcomes of the pilot study.

Since animal health information is being requested, the IACUC recommends that the PIs

consult with the Attending Veterinarian and/or Biomedical Resource Center staff during report preparation to ensure that the reported animal health outcomes are in line with any observations. The IACUC will review reports during the next available convened IACUC meeting.

PROCEDURES FOR PROTOCOL SUSPENSION

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose: This sets for the process by which the IACUC may suspend or terminate an approved project.

Definitions: The IACUC can suspend or terminate any approved research protocol not conducted in accordance with IACUC requirements, university policy, or the AWAR and/or The Guide. The IACUC may also suspend or terminate any approved research protocol that has resulted in serious animal welfare concerns. Suspension is a temporary halt in any ongoing research activities, including collecting new animals or activities involving previously collected animals. Termination is a permanent halt in all ongoing research activities and closure of the project.

Procedure:

1. The convened IACUC may vote to suspend or terminate a previously approved project if they become aware (via either internal or external report) that a project is not conducted in accordance with IACUC approval or requirements. The decision and rationale are documented in the meeting minutes.
2. If the Attending Veterinarian (AV), IACUC Chair, or IO becomes aware (via either internal or external report) that a project has had a significant impact on animal welfare, they may immediately suspend the project until the convened IACUC can meet. The convened IACUC must review the decision and determine by a majority vote whether they will impose a suspension.
3. The IACUC must notify the Institutional Official (IO) when a project is suspended. The IACUC will consult with the IO to review the reason for suspension and determine appropriate corrective actions. The IO may also independently choose to suspend a project, but the project may only be reinstated through a majority vote of the IACUC at a convened meeting.
4. If the IACUC AV or the convened IACUC suspend or terminate a project, the PI must be promptly informed in writing with a statement of the reasons for the suspension or termination.
5. The PI must submit a report to the IACUC with a plan to bring the research back into compliance and ensure the issues will not arise again. The PI must also submit any modifications to the protocol to ensure future compliance.
6. The AV and the IACUC will assist the IO in reporting any suspension or termination decisions to external sponsors and/or regulatory agencies, if applicable to the study.
7. At a convened meeting of the IACUC, the committee will review the proposed action plans, amendments submitted, and a timeline for completion. The IACUC will create a

corrective action plan which will include a determination of which corrective actions need to be in place prior to protocol reinstatement and develop a plan and schedule for the items which must be developed and implemented prior to lifting the suspension. A convened quorum of the committee shall be present for this review to determine whether the measures will satisfy requirements for reactivation or if the protocol should be permanently suspended.

PROLONGED RESTRAINT

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

ACHE Institutional Animal Care and Use Committee

Prolonged Restraint

Definitions:

Physical Restraint - Physical Restraint is defined as "the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation" (The Guide, 2011). Such restraint may cause distress and/or pain if not carried out properly and animals should be acclimated to reduce discomfort. Systems that do not limit an animal's ability to make normal postural adjustments (i.e., tether system) should be used whenever possible.

Purpose:

The Guide does not define "prolonged restraint" and thus tasks the IACUC with defining what constitutes it. The purpose of this Guideline is to define prolonged restraint at ACHE.

Guidelines:

Category 1: Animal restraint in this category permits the animal little or no freedom of movement of the body trunk or limbs and does not permit any change in body position. This form of restraint will be considered "prolonged" when it exceeds 30 minutes in duration.

Category 2: Animal restraint in this category allows considerable freedom of movement of limbs and the animal can change position (e.g., sitting to standing but may not be able to turn around). This form of restraint will be considered "prolonged" when it exceeds 3 hours in duration.

References:

Animal Welfare Act as amended (7 U.S.C. §§ 2131 et. seq.) Title 9, Code of Federal Regulations, Subchapter A, Section 2.31

National Research Council (2011). Guide for the Care and Use of Laboratory Animals. The National Academies Press: Washington, D.C.

Public Health Service Policy on the Humane Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare (OLAW). 1985. Rev. 2015.

PROTOCOL REVIEW PROCESS

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

IACUC (Institutional Animal Care and Use Committee) Review

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

1. Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.
2. Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator.
3. Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure or, if appropriate, during the procedure.
4. The living conditions of animals will be appropriate for their species and contribute to their health and comfort.
5. The housing, feeding, and nonmedical care of the animals will be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied.
6. Medical care for animals will be available and provided as necessary by a qualified veterinarian.
7. Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures.
8. Methods of euthanasia used will be consistent with the current American Veterinary Medical Association (AVMA) guidelines for the Euthanasia of animals unless a deviation is justified for scientific reasons in writing by the investigator.

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

unless they are also voting members of the IACUC. Any use of telecommunications will be in accordance with NIH current policy.

Prior to review, each IACUC member will be provided with written descriptions of activities (protocols) that involve the care and use of animals and any member of the IACUC may request a full committee review (FCR) of those protocols within the five-business day polling period. If FCR is requested, approval of those protocols may be granted only after review at a convened meeting of a quorum of the IACUC and with the approval vote of a majority of the quorum present.

Full-Committee Review (FCR)

All New Protocols undergoing review for the first time and any protocol called to full committee by any voting IACUC member will be reviewed by the full committee. A quorum is required for full committee review of protocols.

The possible outcomes of FCR are as follows:

1. Approval.
2. Require modifications (to secure approval); and
3. Withhold or deny approval

Review of Required modifications Subsequent to FCR. The review of required modifications after FCR will be done in accordance with PHS Notice Number: NOT-OD-09-035, January 2009. When the IACUC requires modifications to secure approval of a protocol, such modifications are reviewed as follows:

1. FCR or DMR following the procedures delineated in the PHS Policy. DMR must be approved unanimously by all members at the meeting at which the required modifications are developed and delineated AND only if the entire current Committee has previously approved, in advance and in writing, e.g. established/approved a written policy, that the quorum of members present at a convened meeting may decide by unanimous decision to use DMR subsequent to FCR when modification is needed to secure approval. However, any member of the IACUC may, at any time, request to see the revised protocol and/or request FCR of the protocol.
2. Minor modifications of an administrative nature, i.e., typographical or grammatical errors, required signatures, etc. may be confirmed by BRC administrative/support personnel.

Designated-Member Review (DMR)

For instances where the IACUC uses the DMR method, the Chair will appoint primary and secondary reviewers via a pre-approved rotating roster. The protocol is assigned to the reviewers and distributed to all other IACUC members to allow all members to call for FCR. The IACUC has five working days for this polling process.

1. If FCR is not requested, the reviewers assigned to the protocol have the authority to approve, require modifications to secure approval, or request FCR of those protocols. If the DMR reviewers do not call for FCR, approval is given only after the designated

reviewers unanimously approve the final version of the protocol form. If the DMR reviewers do not unanimously approve, the protocol is sent to FCR.

2. Other IACUC members may provide the designated reviewer(s) with comments and/or suggestions for the reviewer's consideration only. That is, concurrence to use the designated-member review (DMR) method may not be conditional.
3. Approval of protocols via DMR are maintained, provided to the IACUC in the agenda of the next convened meeting, and recorded in the minutes of the next convened IACUC meeting.
4. The possible outcomes of DMR are as follows:
 - a. Approval of the protocol.
 - b. Require modifications to secure approval and/or that specific conditions be met for final review and approval.
 - c. Send the protocol to the full committee for review.

The IACUC procedures for reviewing proposed significant changes in ongoing research projects are as follows:

Review and approval of significant changes are handled in the same manner as new protocols. Examples of changes considered to be significant include, but are not limited to the following changes:

1. from non-survival to survival surgery.
2. resulting in greater pain, distress, or degree of invasiveness.
3. in housing and or use of animals in a location that is not part of the animal program overseen by the IACUC.
4. in species.
5. in study objectives.
6. in Principal Investigator (PI); and
7. that impacts personnel safety.

Changes of less than 10% or no more than 5 animals of mice of the genus, *Mus* and rats of the genus *Rattus* that are bred for use in research only may, at the IACUC's discretion, be considered minor (not significant).

Veterinary Verification and Consultation (VVC):

The Office of Laboratory Animal Welfare (OLAW) issued a Guidance on Significant Changes to Animal Activities (NOT-OD-14-126) which was developed to support the use of performance standards and professional judgment to reduce the regulatory burden on the Institutional Animal Care and Use Committee (IACUC). The purpose of this guideline is to enable the IACUC to authorize the Attending Veterinarian (AV) to make certain allowable changes to pre-approved IACUC protocols through the process of Veterinary Verification and Consultation (VVC). The VVC process is as follows:

1. All changes to the protocol must be submitted to the IACUC via the amendment process for review.
2. Personnel will administratively review the submission and determine if it meets criteria for VVC based on IACUC approved guidelines and verify that compliance with the VVC

policy is appropriate for the animals. The AV will then confirm whether it qualifies and review the submission. The AV may refer *any* VVC request to the IACUC for FCR review for any reason. In addition, the IACUC will be notified of these types of reviews and have five working days to review the submission and call for full committee. Consultation with the AV will be documented, and the PI will receive written confirmation that the change has been reviewed and approved. If the change does not qualify for VVC as determined by administrative or AV review, it will then be sent through the normal review route as previously described. A list of protocols reviewed and amended by VVC will be provided to the IACUC at the next scheduled IACUC meeting and included in the meeting minutes.

Notify investigators and the Institution in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval according to PHS Policy IV.C.4. The IACUC procedures to notify investigators and the Institution of its decisions regarding protocol review are as follows:

The IACUC will notify investigators and the institution in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval. Principal Investigators are notified by a letter sent electronically. If modifications are required, the letter sent to the PI delineates the required modification(s) needed to secure approval and/or conditions that need to be met for final review and approval. If the IACUC decides to withhold approval of an activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or in writing. The Institutional Official receives a copy of the IACUC meeting agenda and electronic access to all approved meeting minutes which includes a list of all protocols approved by DMR or VVC since the last meeting.

RODENT ANALGESIA

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose

These guidelines describe appropriate analgesia regimens for managing pain in animals used in teaching, research, and testing. Animals should be monitored for an appropriate period to determine if analgesia provisions are adequate. Any animal showing evidence of pain should be provided analgesia. If analgesia cannot be provided due to scientific reasons, the rationale should be described and approved in the Animal Protocol.

Recognition of Pain

Adequate alleviation of pain in laboratory animals requires the training and knowledge to recognize signs of pain which may differ between species. Veterinary consultation is available for personnel training and advice on pain recognition in animal models.

Pre-Emptive Use of Analgesic Agents

Pre-emptive analgesia should be provided whenever possible. Analgesia provisions are most effective at reducing the intensity of painful stimulation when given prior to the painful event.

1. Advantages of pre-emptive use of analgesics:
 - a. Reduces the intensity of painful stimulation
 - b. Improves the animal's comfort level after surgery
 - c. Decreases the amount of anesthesia required to maintain a surgical plane
 - d. Results in a smoother recovery

To comply with regulatory requirements, the ACHE Institutional Animal Care and Use Committee (IACUC) has in effect the following guidelines:

Rat Analgesics

Name	Dosage	Route(s)	Frequency
Buprenorphine (see comments below in different formulations)	0.01 - 0.05 per 3 rd ed	SC, IV	Every 8 hours
Carprofen	5 mg/kg	SC, PO	Every 12 hours (every 24 hours if using PO Bioserv tablets)
Meloxicam	1-2 mg/kg	SC	Every 24 hours

Mouse Analgesics

Name	Dosage	Route(s)	Frequency
Buprenorphine (see comments below in different formulations)	0.05 - 0.1 mg/kg	SC	Every 8 hours
Carprofen	5 mg/kg	SC, PO	Every 12 hours (every 24 hours if using PO Bioserv tablets)
Meloxicam	2-5 mg/kg	SC	Every 24 hours

Buprenorphine (Buprenex®) General Info:

1. a partial mu opiate agonist
2. a Schedule III controlled substance
3. primary form: injectable **Recommended Dose/Route/Frequency**

Mouse:

0.05-0.1 mg/kg, Subcutaneous, every 8 hours

Rat:

0.01-0.05 mg/kg, Subcutaneous or Intravenous, every 8 hours

0.5 mg/kg, by mouth, every 12 hours

Buprenorphine in Extended-Release Polymer (Wedgewood Connect Generic)

General Info:

1. a sustained release buprenorphine that will last up to 72 hours with a single injection
2. comes in concentrations of 0.5mg/ml for mice and 1.0 mg/ml for rats

Recommended Dose/Route/Frequency

Mouse:

1.5 mg/kg, Subcutaneous

Rat:

0.5 mg/kg, Subcutaneous

Buprenorphine Extended-Release Suspension (EthiqXR®) 1.3mg/ml

General Info:

1. FDA-approved pharmaceutical grade product
2. May be re-dosed at 72 hours

Recommended Dose/Route/Frequency

Mouse:

3.25 mg/kg, Subcutaneous

Rat:

0.65 mg/kg, Subcutaneous

Note on Oral Administration {PO}

In rodent species, historically, the use of analgesics such as Ibuprofen (Children's Advil® Elixir) has been administered in drinking water for post-surgical procedures. This was performed based on the assumption that continuous administration of the drug by consumption in the water would provide a hands-off, stress-free, continuously administered level of analgesic therapy.

With continued investigation, it has been demonstrated that water and food consumption post surgically and/or post-anesthesia are neither constant nor consistent (1-4). As a result, analgesics may not be consumed by the animal. "Confirmed administration" is encouraged by routes such as injection or oral/gastric gavage to ensure that the animal received the appropriate dose of medication to better manage discomfort. Administration of analgesics via drinking water requires justification and special approval by the IACUC.

Opioids: Buprenorphine

Opioid drugs produce their effect by binding three different receptors [μ (μ), kappa (K), and delta (δ)] as either agonists, partial agonists, or antagonists. The location of these receptors varies, but in general, reside within the brain and spinal cord.

1. Advantages: Provide potent analgesia; concurrent administration can lower the dose of inhalant or barbiturate general anesthetic for surgery; mechanism mediated by receptor binding in the brain and spinal cord; long history of use in research.
2. Disadvantages: DEA Controlled Class II-IV drugs; high potential for human abuse and addiction; short duration of action; repeated use may result in tolerance development.
3. Additional Notes: Duration of effect has continuously hampered the use of opioids in research animals. In general, opioids are short acting drugs.

Non-steroidal Anti-Inflammatory Drugs {NSAIDs}: Carprofen, Meloxicam, Ibuprofen

Members of this group represent 13 different classes of drugs which share inhibitory activity of the cyclooxygenase (COX) enzyme. The COX enzyme facilitates the production of Prostaglandin G₂ (PGG₂) which then follows a variety of enzymatic processes in the production of several compounds that are involved in normal physiological processes and production of Prostaglandin E₂ (PGE₂). PGE₂ specifically plays a role in the perception of pain in the periphery and within the central nervous system. Thus, blockade of PGE₂ by COX inhibition is effective in control of discomfort at the site of insult and within the central nervous system. Two forms of the COX enzyme have been well characterized (COX-1 and COX-2). As a result, COX inhibitors are often referenced as non-selective COX inhibitors or selective COX-2 inhibitors.

This distinction has been made because inhibition of COX-2 is believed to be the predominant method of NSAID function to provide analgesia and anti-inflammatory action even though this "consensus" is still under debate. Over the past 10 years, several NSAIDs have emerged for veterinary use that are COX-2 selective, such as Carprofen and Meloxicam which can be administered once every 12-24 hours in most species.

1. Advantages: New drugs (Carprofen, Meloxicam) including a long duration of analgesic activity; newer drugs demonstrate analgesic quality that rivals some opioids; not a DEA controlled substance; there are multi-route administration methods for several NSAIDs; relative safety when administered at prescribed dosages.
2. Disadvantages: Contraindicated for inflammation models, infectious disease, or coagulation research due to anti-inflammatory properties; COX-1 side effects such

as: gastrointestinal complications, prolonged coagulation times, and changes in kidney function with non-COX-2 selective forms.

3. Additional Notes: Analgesic combinations that include NSAIDs plus opioids would be considered an ideal combination for the control and prevention of discomfort due to the demonstrated harmony and difference in mechanism of action. In contrast, it is discouraged to combine multiple NSAIDs in combination or use NSAIDs in combination with steroids (Prednisone, Prednisolone, and Dexamethasone) as the incidence of complications increases.

RODENT ANESTHESIA

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

To comply with regulatory requirements, the ACHE Institutional Animal Care and Use Committee (IACUC) has in effect the following Guidelines:

Terms

Abbreviation	Definition
IP	Intraperitoneal
SQ	Subcutaneous
IT	Intrathecal
INH	Inhalation
IM	Intramuscular
DEA	Drug Enforcement Administration
NSAID	Non-steroidal anti-inflammatory drug
IACUC	Institutional Animal Care and Use Committee
GABAA	γ -Aminobutyric acid sub-type A

Rat Anesthetics

Type	Dosage	Route(s)	Duration
Isoflurane	Induction: 3-5% Maintenance: 1.5-3%	INH,IT	Ongoing
	Maintenance: 1.5-3%		
Ketamine/Xylazine	75/10 mg/kg	IP	20-40 minutes
Ketamine/Xylazine Acepromazine	75/5/1 mg/kg	IP	30% longer duration and recovery than ketamine/xylazine alone
Pentobarbital	30-60 mg/kg	IP	20-40 minutes

Lidocaine (1-2%)	2-4 mg/kg (max 7 mg/kg)	SQ/topical for local anesthesia only	30-60 minutes (5–10-minute onset)
Bupivacaine (0.5%)	1-2 mg/kg (max 8 mg/kg)	SQ/topical for local anesthesia only	4-8 hours (15–30-minute onset)

Mouse Anesthetics

Type	Dosage	Route(s)	Duration
Isoflurane	Induction: 3-5% Maintenance: 1.5-3%	INH, IT	Ongoing
Ketamine/Xylazine	80-100/6-10 mg/kg	IP, IM	20-40 minutes
Ketamine/Xylazine Acepromazine	65/13/2 mg/kg	IP	30% longer duration and recovery than ketamine/xylazine alone
Pentobarbital	40-90 mg/kg	IP	20-40 minutes
Lidocaine (1-2%)	2-4 mg/kg (max 7 mg/kg)	SQ for local anesthesia only	30-60 minutes (5–10-minute onset)
Bupivacaine (0.5%)	1-2 mg/kg (max 8 mg/kg)	SQ for local anesthesia only	4-8 hours (15–30-minute onset)

Neonatal Rodent Anesthetics

Neonates are defined as mouse or rat pups up to 10 days of age. Gas anesthesia (isoflurane) is recommended for longer, more invasive procedures in neonates older than five days.

Ketamine/xylazine may be considered for use in neonates but may not be as safe as isoflurane. It is extremely important to accurately weigh the animal and calculate the correct dose. Starting with the lower dose range is recommended. Recovery period may be prolonged. Repeat administration is not advisable.

Type	Dosage	Route(s)	Max.	Duration
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Isoflurane	Induction: 4-5% Maintenance: 1-2% Recovery: 0.5% then 0	INH, IT	N/A	Ongoing
Ketamine/Xylazine	Mouse: 50-150/5-10 mg/kg	IP <27g	<0.5 mL	20-40 minutes
	Rat: 40-90/5-10 mg/kg	IP <25g	<1.0 mL	20-30 minutes
Cryoanesthesia may be used if pups are < 6 days old	Place pups on crushed ice covered with aluminum foil so they do not contact the ice	N/A	N/A	8-10 minutes; warm slowly on a water recirculating heating pad to avoid tissue damage

Note: Other anesthetic regimes may be acceptable with veterinary and IACUC approval
Drug Considerations

Isoflurane (Forane®, Iso, IsoFlo®): Isoflurane is the first choice of anesthetic used for animal restraint or surgical procedures in laboratory animal species. Isoflurane is administered to rodents through an intratracheal tube or inhaled via a nosecone. The concentration of drug can be administered to effect by adjusting the percent of displacement of O2 with a precision vaporizer. Maintenance anesthesia is typically between 1.5-3% isoflurane. Induction of anesthesia with gas is typically achieved with <2 min exposure to 3-5% isoflurane.

- Advantages: Rapid induction and recovery; a precision vaporizer can precisely titrate the level of anesthesia during a procedure; liquid isoflurane is not a DEA controlled drug.
- Disadvantages: Upfront cost associated with a precision vaporizer; requires either passive or active scavenging of waste and exhaled anesthetic gas; occupational health exposure to anesthetic gases should be limited; prolonged analgesic effect is not achieved after the animal is awake; depressed respiratory rate and decreased blood pressure.
- Additional details:
 - Advantages typically outweigh disadvantages as gas anesthesia is the first recommendation for anesthetic administration due to rapid induction, recovery, and precise dose titration during the procedure.
 - The duration of anesthesia can be easily adjusted for a variety of procedures ranging from 30 seconds up to many hours.
 - Concurrent use of analgesics such as opioids or NSAIDs is encouraged as Isoflurane has no analgesic properties once the animal is awake from the procedure.
 - Occupational exposure is always a concern. Gas anesthesia must be vented from the room (table-top back-draft vents, biosafety cabinet with 100% exhaust outside the building) or filtered through passive scavenging using F/Air®

activated charcoal canisters. F/Air® canisters must be weighed on a very regular basis and replaced before the canister gains 50 grams of weight during use. Isoflurane exposure levels should be monitored annually by EHS.

- If using the drop method, the animal must not contact the liquid anesthetic.

Ketamine (Ketaset®): Ketamine is the most used injectable anesthetic for a variety of species. However, ketamine used as the sole anesthetic is not recommended. In most cases, ketamine is used in combination with other injectable agents such as $\alpha 2$ agonists (xylazine) or benzodiazepines to reduce or eliminate many of the less desirable side effects if used alone. In rodents, ketamine combined with xylazine or xylazine plus acepromazine are the preferred anesthetics when gas anesthesia cannot be used.

- **Advantages:** Ketamine has a wide margin of safety in most species; residual analgesic effect following anesthetic recovery, most used drug (in combination) for injectable anesthesia in rodents.
- **Disadvantages:** Ketamine alone does not provide muscle relaxation and muscle spasms may be observed; DEA license required for use as ketamine is a class III-controlled substance; surgical anesthesia may be limited depending on the species; prolonged recovery as compared to gas anesthetics (true for any injectable anesthesia).
- **Additional details:**
 - **Ketamine + xylazine:** Both drugs can be mixed in a single syringe prior to administration (with the completion of a non-pharmaceutical grade compound appendix in the corresponding IACUC protocol). This combination is the most common injectable anesthetic used in rodent species.
 - **Ketamine + xylazine + acepromazine:** All three drugs can be mixed in a single syringe prior to administration (with the completion of a non-pharmaceutical grade compound appendix in the corresponding IACUC protocol). In rodents, the addition of acepromazine to the ketamine + xylazine cocktail increases the depth of anesthesia and prolongs the duration of anesthesia as well as recovery time. The benefit of this combination will be dependent on the procedure.

Xylazine (Rompun®): Alpha-2 agonists are used for their sedative and analgesic properties in a variety of species. Used as the sole agent, they do not produce an adequate level of anesthesia for even minor surgical procedures. However, in combination with ketamine, $\alpha 2$ -agonists become much more useful and effective as anesthetics for surgical procedures.

- **Advantages:** Produces analgesia of short duration; can be combined with ketamine to produce adequate surgical anesthesia in many species; effects can be reversed with a subcutaneous $\alpha 2$ antagonists.
- **Disadvantages:** Cardiovascular depression (decreased heart rate, cardiac output, and hypotension); transient hyperglycemia following administration which may have research significance.
- **Additional details:**
 - When re-dosing an injectable anesthetic combination of ketamine and an $\alpha 2$ agonists as the initial injection is wearing off, it is recommended to only re-dose

ketamine as the duration of action of the α_2 agonist is much longer than the duration of effect of ketamine.

Sodium Pentobarbital (Nembutal®): Barbiturates function as GABA_A agonists and are good anesthetic agents but provide unreliable sedation at low dosages and inadequate analgesic effect at any dose. Pentobarbital, the most used drug of this class, is considered a long-acting anesthetic.

- Advantages: Rapid anesthetic onset; prolonged duration of surgical anesthesia; decades of use have characterized many research side effects.
- Disadvantages: Prolonged recovery time; inadequate analgesic properties; extremely expensive; narrow margin of safety; produces respiratory depression at higher dosages; DEA License required for use as a class II-controlled substance.

Eye Protection

Rodents' eyes remain open under anesthesia. This can lead to corneal drying and trauma. Apply ophthalmic ointment (e.g., Paralube® Lacrilube®) to eyes if:

1. The anesthetic event lasts longer than two minutes.
2. Anesthesia is being delivered by facemask.

Acclimation period and health observation: To avoid stress following vehicle transportation, animals should be acclimated for at least 2 days before general anesthesia and 4 days before major survival surgery. 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.

SEMI-ANNUAL INSPECTIONS

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose

ACHE is committed to observing Federal policies and regulations and the Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC) International standards for the humane care and use of animals. This document describes the ACHE Institutional Animal Care and Use Committee (IACUC) requirements and process for conducting semiannual inspections. Adherence to this policy is mandatory unless a specific exception has been approved by the IACUC.

Covered Parties

These guidelines are applicable to all persons responsible for conducting research, teaching, training, breeding, and related activities, hereinafter referred to collectively as “activities,” involving live vertebrate animals conducted at or under the auspices of ACHE.

Regulatory Requirements

The [Animal Welfare Act \(AWA\) and associated regulations](#) and [Public Health Service \(PHS\) Policy](#) require IACUCs to inspect an institution’s animal facilities at least once every six months. This includes areas where United States Department of Agriculture (USDA)-regulated species are maintained for >12 hours, satellite facilities where non-USDA regulated species are maintained for >24 hours, transport vehicles, and areas where surgical manipulations (minor, major, survival, or non-survival) are performed.

Areas containing free-living wild animals in their natural habitat (field study sites) are exempt from semiannual inspections; however, IACUCs should be apprised of the circumstances under which studies are conducted so that they can consider risks to personnel and impact on study subjects.

The following flexibilities are provided in the PHS Policy & AWA and regulations:

1. The timing of facility inspections may extend up to 30 days beyond the six-month interval from the last review if there is no forward drift of the date from year to year.
2. The IACUC has discretion to determine the best means of conducting facility inspections. This includes using qualified individuals (i.e., IACUC members, IACUC Staff, or veterinarians) and appropriate *ad hoc* consultants.
3. ACHE IACUC must use subcommittees of at least two voting committee members but may invite *ad hoc* consultants to assist in the inspections.
4. No Committee member wishing to participate in IACUC inspections may be excluded. The IACUC remains responsible for the inspections and semiannual reports to the Institutional Official.

Protocol Requirements

The use and/or housing of animals outside the centralized animal facility must be described and justified in an approved IACUC protocol. The IACUC office will verify which of the review methods below is required:

If a location needs to be added and it is already included in the current animal program, then it can be administratively approved. If a location needs to be added that is not included in the current animal program, it must be inspected by two voting members and approved by a valid IACUC review method (DMR or FCR).

IACUC Inspection Process

Every facility in which animals are housed for more than 12 hours will be inspected twice a year, 6 months apart, by at least 2 voting members of the IACUC.

Transportation vehicles

Vehicles used to transport animals are subject to the same inspection requirements as animal facilities. They will be inspected at least semiannually by at least 2 members of the IACUC.

Surgery – minor, major, survival, or non-survival

Every location in which animals are used in surgical procedures must be inspected twice a year, 6 months apart, by at least 2 voting members of the IACUC.

Note: NEW housing and surgical locations not included in the current animal program require IACUC approval and inspection before use.

Other activities

Areas utilized for other activities (such as routine weighing, dosing, immunization, imaging, euthanasia/tissue harvest, etc.) will be inspected every 6 months during semiannual inspections.

Areas not located on ACHE campuses (e.g., field studies, community school-age activities) may undergo alternative means of conducting semiannual inspections (virtually or pictures/videos) instead of in-person inspections.

New IACUC members will be trained in conducting Semi-Annual Inspections.

Additional Institutional Regulatory Group Involvement

During IACUC inspections, committee members may note potential issues that fall under the purview of other institutional regulatory groups or offices, (e.g., Institutional Biosafety Committee) If this occurs, the information will be communicated to the appropriate party/parties outside of the IACUC Inspection Report. This communication is advisory only and does not confer responsibility on the IACUC.

Related Policies and References

- [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#)
- [Animal Welfare Act and Animal Welfare Regulations](#)

- [Guide for the Care and Use of Laboratory Animals: Eighth Edition](#)

SOCIAL HOUSING AND ENRICHMENT

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Environmental Enrichment:

The Guide for the Care and Use of Laboratory Animals states: “The primary aim of environmental enrichment is to enhance animals’ 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”¹.

All animals will receive enrichment unless specifically justified by the primary investigator and approved by the IACUC. Animals will be provided with at least one enrichment item. Animals will receive enrichment each time the cage is changed.

Items that may be used for enrichment need to be evaluated for use by considering the following:

1. The species of animal (its needs, habits, and capabilities)
2. The type of enrichment device (treat, exercise, toys)
3. The utility of the device (its ability to stimulate the animals’ interest)
4. The safety of the device (not injurious to the animal and its ability to be sanitized for future use)
5. The nature of the research being done (research will not be compromised due to placement of an enrichment device)
6. Cage complexities and important resources (perches/shelves, visual barriers, refuges, food, water, shelter, and enrichment devices) should be provided in such a way that cannot be monopolized by dominant animals or elicit aggression between animals.

Housing and Cage Density:

The Guide for the Care and Use of Laboratory Animals states: “The need for single housing based upon experimental requirements is the exception and must be scientifically justified in the animal use protocol and reviewed and approved by the IACUC.”²

Single housing of social species should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being. The need for single housing should be reviewed every 30 days if due to veterinary intervention and annually if approved as part of the IACUC protocol.

Items to consider for housing:

1. The risks of social incompatibility are greater when introducing adult animals so consideration should be given to introducing adult animals at a younger age to reduce aggressive interactions. The social stability of newly created groups should be carefully monitored for excessive aggression and incompatible individuals separated.
2. When single housing is justified it should be limited to the minimum period necessary, and where possible, visual, auditory, olfactory, and tactile contact with compatible conspecifics should be provided. In the absence of other animals other forms of enrichment should be offered such as positive interaction with staff and additional enrichment items.

In general, the number of animals allowed per cage/enclosure is based on the Guide's mathematical formulas. In some cases, animals may require more space than the minimum listed in the Guide.

Enrichment by Species Housed:

Rats (*Rattus norvegicus*)

1. Social housing – Rats are pair or group housed unless the requirements of the research protocol, or animal health concerns make this impossible. Any exception to pair or group housing must be approved by the IACUC. Additional enrichment is recommended for single housed animals.
2. Structure and substrate – In addition to the caging substrate, additional nesting materials such as strips of paper fibers may be requested by the investigator.
3. Manipulanda/toys – Nylabones, wooden, or other chew toys used for gnawing are the standard enrichment for rats.
4. Single Housed Rats: Additional Nylabones, and cardboard or PVC tube should be added.
5. It is recommended that young rats be exposed to the practice of “rat tickling” to decrease stress responses when being handled or manipulated.

Mice (*Mus musculus*)

1. Social housing – Mice are pair or group housed unless the requirements of the research protocol or animal health concerns make this impossible. Any exception to pair or group housing must be written in the animal use protocol and approved by the IACUC. Extra enrichment (e.g., tunnels, huts, wheels) is recommended for single housed animals.
2. Structure and substrate – Nest building material is provided allowing the expression of nesting behaviors. Exceptions to the use of the standard Enrichment must be requested approved by the IACUC.
3. Manipulanda/toys – Additional items such as paper or plastic housing structures, or running wheels may also be provided if approved by the PI (Principal Investigators)
4. It is recommended that mice be provided with a tunnel to facilitate tunnel handling.

Fish

1. Social housing – Fish are housed in social or breeding groups at an appropriate stocking density.

2. Structure and substrate – Fake substrate in the form of a picture mounted beneath the tank, plastic plants, marbles, mirrors, and other commercially available tank inserts may be provided.
3. Singly housed fish – it is recommended singly housed fish be provided with extra enrichment

SURGERY (NONUSDA SPECIES)

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose

This guideline is to guide personnel conducting survival surgical procedures on species not covered by the USDA Animal Welfare Act.

The *Guide for the Care and Use of Laboratory Animals* (NRC 2011) states, "For most survival surgery performed on rodents and other small species such as aquatics and birds, an animal procedure laboratory, dedicated to surgery and related activities when used for this purpose and managed to minimize contamination from other activities conducted within the room at other times, is recommended."

Limiting nonsurgical activities in the laboratory may help to minimize contamination of the surgical area. However, minimizing contamination during surgery may be achieved by considering several factors. The specific location of the surgical area within the laboratory should promote the proper conduct of sterile technique, and to the extent possible, it should be isolated from other activities in the laboratory. The surgical area should be dedicated for that purpose while surgery is performed. Other factors that may impact the risk of contamination include the invasiveness and complexity of the surgical procedure, duration of surgery, and the nature of other non-surgical activities conducted in the laboratory (i.e., their likelihood of increasing the risks of surgical contamination). For complex or long procedures, or if the layout of the laboratory does not permit a suitable dedicated surgical space, it may be advisable to temporarily stop other laboratory activities, thereby dedicating the laboratory to surgery to maximize the potential for a good surgical outcome. For minor surgeries of short duration, conducted in a suitable area within the laboratory, it may be acceptable to allow other laboratory activities to continue if they do not jeopardize aseptic technique. The investigator, IACUC (or comparable oversight body) and veterinarian should evaluate surgical areas to ensure they are appropriate.

Survival Surgery

All survival surgical procedures performed on non-USDA-covered species must follow the general principles of asepsis. Aseptic technique is used to reduce microbial contamination to the lowest practical level which includes preparation of the patient, surgeon, and instruments; and requires adherence to certain practices throughout the procedure.

Surgical Space

Surgery on animal species not covered by the USDA does not have to be performed in a dedicated surgical suite. A procedure room within the animal facility or a dedicated space within the lab may be used. If the space is within the lab, it must be done in an area dedicated to surgery.

The space should be chosen based on space, ease of sanitization, and traffic flow. The space should be large enough to designate separate areas for animal preparation, surgery, and recovery. The area should also be uncluttered and clean. It should have hard, impermeable surfaces that may be easily sanitized before and after surgery. Because the main source of intraoperative infection is airborne bacteria, the area should have minimal traffic to decrease the risk of infection. This surgical space must be described in the IACUC protocol and requires IACUC approval and semi-annual IACUC inspections.

Remove any extraneous materials from the surgical space and clean the area with a disinfectant. Prior to surgery and collecting the animals, gather and aseptically lay out all of the necessary supplies and equipment, including sterilized instruments/implants, drapes, suture material, anesthetics, and analgesics. Set up the surgical location with a warming device and proper lighting.

Preparation of the Animal

If possible, animal preparation should occur in an area separate from the operating space and after the animal is sedated/anesthetized. Preparation must include the following:

1. Placing ophthalmic ointment in both eyes to prevent drying of the cornea during surgery.
2. Removal of hair/feathers from the surgical site using clippers or depilatory cream. Feathers may be manually removed. The shaved/plucked area should be wide enough to avoid contamination from the surrounding skin and hair/feathers during surgery but should be the minimum compatible with achieving an appropriate sterile field. Removing more hair/feathers than is necessary predisposes the animal to hypothermia during the surgical procedure. Hair/feathers that have been removed, along with hair removal cream if used, should be cleaned away. If using a depilatory cream, it is important to be sure to thoroughly remove all of it prior to surgery to avoid chemical burns. Following completion of the above procedures, the animal should then be moved to the surgical field. Once the animal has been moved, preparation of the animal is continued.
3. The skin must be disinfected using a concentric surgical scrub pattern moving from the innermost to outermost portions of the shaved area. Scrub the area no less than three alternating scrubs with antiseptic followed by alcohol
4. Apply a sterile drape

Preparation of the Surgeon and Surgical Assistant(s)

Surgeons and surgical assistants who will have contact with the sterile surgical field during the surgery must prepare in the following manner:

- Surgeons must use appropriate personal protective equipment (PPE) for the species and Animal Biosafety Level (ABSL). The minimum PPE includes a clean lab coat or disposable gown, an appropriate face mask, a hair bonnet, and closed toed shoes. The face mask and hair bonnet must be donned prior to surgical gloves.
- The surgeon should thoroughly wash their hands and wear sterile surgical gloves of the correct size donned using the appropriate technique. If a non-sterile item/surface is accidentally touched, the surgeon must change the contaminated gloves for a new sterile pair.

- Non-surgeons that will not have contact with the sterile field must still wear appropriate PPE, including a disposable cover gown, a mask, a hair bonnet, and exam gloves.

Preparation and Handling of Surgical Instruments, Equipment, or Implanted Material, and Consumables

- All surgical instruments must be cleaned and autoclaved prior to use on animals for all survival surgical procedures.
- Autoclaved surgical packs should contain a sterilization indicator. The date of sterilization and the expiration date should also be listed on the pack
- Equipment or implants that will encounter the sterile field that are unable to withstand the conditions of autoclaving must be sterilized in another way.
- If surgeries are completed on multiple animals in the same session, a hot bead sterilizer can be used to sterilize the instruments between animals. It is important to use the hot bead sterilizer correctly to ensure sterilization. **No more than 8 surgeries** may be performed with instruments using the hot bead sterilizer. After that point, a new autoclaved pack must be used.
- Instruments should be placed on a sterile surface (e.g., sterile drape) when not being used to avoid accidental contamination of the surgical site.
- When it is suspected that instruments may have been accidentally contaminated, these must be replaced with sterile ones before continuing.

Intraoperative Monitoring

Animals must be monitored throughout the duration of the surgery to ensure an adequate plane of anesthesia using measures appropriate for the species. This must be done and documented every 15 minutes. The animal must be continuously monitored until fully recovered from anesthesia. Recovery is indicated by normal ambulation and behaviors. Only when an animal is fully recovered can it be returned to its normal housing location. Complete and place a Surgical Care card on the cage. (Cards are provided by the BRC.) Analgesics, post-operative monitoring, and suture removal must be provided as stated in the approved IACUC protocol.

Recordkeeping

Records documenting any survival surgery and post-operative monitoring must be retained by the lab and be readily available for review by the IACUC, the veterinary staff, and representatives of regulatory and accrediting organizations. All non-USDA-regulated animal anesthesia and surgery records must be retained for three years after cessation of the project.

The following information must be included in the surgical records.

1. Principal investigator name and protocol number
2. Title or brief description of procedure performed
3. Species, identification, and the total number of animals
4. Name of the surgeon
5. Date of the surgery

6. Name and dose/dosage of all agents administered before, during, and after anesthesia and/or surgery, including anesthetics, analgesics, therapeutics, and any experimental agents delivered
7. Monitoring of vital signs
8. Any complications (e.g., respiratory distress, bleeding, prolonged recovery, unanticipated mortality) that occurred during or after the procedure

See Rodent SURGERY monitoring Record (Survival Surgery) under myACHE – BRC site as an example form that can be used as-is or modified for use with specific surgical procedures

The following information must be included in the post-operative monitoring records:

1. Monitoring the integrity of any surgical incisions, ensuring that they are clean, dry, and intact.
2. The animal's general posture and activity.
3. Hydration status and appetite – urine and feces should be present in the cage and indicate that hydration and appetite are normal. A hydrated mouse has sunken eyes and may have pale ears.
4. Additional comments for any variations from the normal and expected events during the recovery period.
5. Analgesics used to include the time, dosage, and route.
6. The initials of the person doing the monitoring.

The frequency of monitoring must adhere to what is described in your approved IACUC protocol. Note: The length of monitoring noted in the protocol is minimum. If there is any indication that the animal is not doing well, the record must extend beyond this period.

A sample Rodent Surgical and Post-Surgical Monitoring Form is available on the BRC myACHE site.

Non-survival Surgery

For non-survival surgeries, it may not be necessary to follow all the requirements outlined for survival surgeries. However, for procedures of extended duration, attention to aseptic technique may be more important to ensure stability of the model and a successful outcome.

The following guidelines describe the minimum requirements for non-survival surgery on non-USDA-regulated species.

Surgical Space

If possible, animal preparation should occur in an area separate from the operating space and after the animal is sedated/anesthetized. Surgery on animal species not covered by the USDA does not have to be performed in a dedicated surgical suite. A procedure room within the animal facility or a dedicated space within the lab may be used. If the space is within the lab, it must be done in an area dedicated to surgery. The space should be chosen based on space, ease of sanitization, and traffic flow. The space should be large enough to designate separate areas for animal preparation, surgery, and recovery. The area should also be uncluttered and clean. It should have hard, impermeable surfaces that may be easily sanitized before and after surgery. Because the main source of intraoperative infection is airborne bacteria, the area should have

minimal traffic to decrease the risk of infection. This surgical space needs to be described in the IACUC protocol and requires IACUC approval and semi-annual IACUC inspections.

Preparation of the Animal

The hair/feathers around the surgical site should be removed and the area should be clean and free of gross debris. Removal may be achieved with clippers, depilatory cream, or by manual plucking for feathers.

Preparation of the Surgeon and Surgical Assistants

Don appropriate personal protective equipment (PPE) for the species and Animal Biosafety Level (ABSL). The minimum PPE includes a clean lab coat or disposable gown, an appropriate face mask, a hair bonnet, closed-toe shoes, and exam gloves. The surgeon does not have to don additional sterile attire but needs to wear clean gloves.

Preparation and Handling of Surgical Instruments, Equipment, or Implanted Material, and Consumables

Instruments should be clean and free of gross debris. Nonsterile instruments and supplies are acceptable. It **is not acceptable** to use any expired goods or materials. If multiple animals are being done on a single day, the instruments need to be cleaned between animals.

Intraoperative Monitoring

Animals must be monitored throughout the surgery to ensure an adequate plane of anesthesia as appropriate for the species. This must be done and documented every 15 minutes

Surgical Recordkeeping

The following information must be included in the surgical records:

1. Principal investigator name and protocol number
2. Title or brief description of procedure performed
3. Species, identification, and the total number of animals
4. Name of the surgeon
5. Date of the surgery
6. Name and dose/dosage of all agents administered before and during anesthesia and/or surgery, including anesthetics, analgesics, therapeutics, and any experimental agents delivered
7. Monitoring of vital signs
8. Any complications (e.g., respiratory distress, bleeding, or unanticipated mortality) that occurred during the procedure
9. How the animal was euthanized at the end of the procedure, including primary and secondary methods

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

1. Included in and essential components of a single research project or protocol
2. Scientifically justified by the Investigator, or

3. Necessary for clinical reasons

The IACUC will pay attention to animal well-being through continuing evaluation of outcomes. Cost savings alone is not adequate reason for performing multiple major survival surgical procedures. Note that some procedures characterized as minor may induce substantial post-procedural pain or impairment and should similarly be scientifically justified, if performed more than once in a single animal.

Note: Surgical procedures are categorized as major or minor. Whether a surgical procedure is major or minor should be evaluated on a case-by-case basis, as determined by the veterinarian and the IACUC. However, generally, a major surgery is any surgical intervention that penetrates and exposes a body cavity or produces permanent impairment of physical or physiologic functions. A minor surgical intervention does not expose a body cavity and/or causes little or no physical impairment.

TRANSPORTATION

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Purpose:

This document establishes ACHE (Arkansas Colleges of Health Education) standards for transportation of animals to ensure the highest level of animal welfare and compliance. It has been developed to ensure that ACHE complies with the Guide for the Care and Use of Animals, 2011, 8th Edition, The Animal Welfare Act and Regulations, and Fish and Wildlife Service laws and regulations related to the transportation of live animals.

Regulatory or Accreditation Authority:

9 C.F.R. Part 3, 3.13 – 3.19, 3.35 – 3.41, 3.60 – 3.66, 3.86 – 3.92, 3.112 – 3.118, 3.136 – 3.142.

(Transportation standards for several types and species of animals, Animal Welfare Regulations)

Guide for the Care and Use of Laboratory Animals, Eighth Edition, 2011, p. 105 – Veterinary Care, Transportation of Animals.

Scope

This applies to all animals transported within ACHE, animals transported to other institutions from ACHE, and animals transported from other institutions to ACHE by ACHE transportation. The scope of this document does not cover animals under the care of commercial shippers. Commercial shippers, when used, assume full responsibility for health, safety, and regulatory compliance for animals in their possession. The BRC (Biomedical Resource Center) maintains a list of veterinary approved commercial shippers.

Procedures

ACHE animals may be transported between buildings and/or to non-ACHE locations using an IACUC (Institutional Animal Care and Use Committee) approved ACHE owned and operated transportation vehicle or by means of approved shipping courier. Animals must not be transported by any other mode of public or private transportation. Transportation parameters must comply with the Animal Welfare Act regulations when applicable. Deviation from this guideline requires IACUC approval.

1. General
 - a. Animals are to be transported via an IACUC approved transport method.
 - b. Animals must not be visible to the public.
 - c. Transportation time should be minimized.

- d. Animals must be transported in enclosures appropriate for the species.
 - e. Enclosures used to transport live animals must not be handled in any manner which may cause physical trauma or stress to the animals.
 - f. Occupational exposure to allergens and infections must be minimized.
 - g. Cages or containers used for transportation and/or shipping must meet minimum standards for size, ventilation, strength, sanitation, and be designed for safe handling. Temperature and Humidity must be monitored and maintained at a species appropriate level per AWA and The Guide. (Guide for the Care and Use of Laboratory Animals, 8th Ed, 2011 and U.S. Animal Welfare Act and Animal Welfare Regulations)
 - h. Cages should be secured so that they will remain upright and safe and optimize ventilation during transport.
 - i. Enclosures which are not disposable must be cleaned and sanitized between each use.
 - j. Animals may not be left unattended.
 - k. Additional requirements may be needed based on species being transported and other considerations (e.g., Bite/Scratch kit; euthanasia solution).
2. Rodent Transportation
- a. Cage tops must be securely fastened.
 - b. Water bottles should be turned upside-down during transport.
 - c. Gel packs and food should be provided in each cage if transport is expected to take longer than four hours.
 - d. Cages are to be covered with or placed inside opaque bags, so animals are not visible to the public.
 - e. Cages should not remain stacked, or in bags, longer than the time needed to reach the destination.
3. Aquatic Transportation
- a. Fish
 - i) Adult fish should be fasted 24 hours before packaging to reduce waste accumulation.
 - ii) Animals should be transported in plastic bags at a density of 2 adult fish/0.5 liter
 - iii) Bags should be securely closed (i.e.: rubber band)
 - iv) Bags should be placed in an insulated shipping box (i.e.: Styrofoam box) and care should be taken to avoid jostling.
 - v) For shorter transportation (housing room to procedure space within the same building), fasting is not necessary and fish may be transported in fish tank with secure lid or secondary container and on a transport cart.
4. Vehicle Transportation
- a. Protocol and Training Requirements
 - i) Non-ACHE vehicles must be approved in the IACUC protocol.
 - ii) Driver must be approved to use an ACHE vehicle
 - iii) A logbook is required for vehicle transport.
 - (1) Log should include driver name, date, starting location and destination, temperature range (min/max), sanitation frequency for vehicle, number of animals, start and end time of trip.
 - (2) Ambient temperature should be regulated by air-conditioning or heating

elements within the vehicle, to maintain temperatures specific to each species.

- (3) The temperature of the animal holding compartment must be monitored and should remain consistent with recommendations for the species transported.
- (4) A contingency plan must be in place in the event of a vehicular accident.

The Attending Veterinarian or designee is responsible for oversight of animal transport and may grant exceptions when deemed in the best interest of the animals. Concerns regarding animal transportation will be reviewed by the IACUC.

VETERINARY VERIFICATION AND CONSULTATION

Approved: 8/29/24

Last Reviewed: 8/29/24

Revision History:

Background

The Office of Laboratory Animal Welfare (OLAW) issued a Guidance on Significant Changes to Animal Activities (NOT-OD-14-126) which was developed to support the use of performance standards and professional judgment to reduce the regulatory burden on the Institutional Animal Care and Use Committee (IACUC). The purpose of this guideline is to enable the IACUC to authorize the Attending Veterinarian (AV) to make certain allowable changes to pre-approved IACUC protocols through the process of Veterinary Verification and Consultation (VVC).

Definitions

Therapeutic Substance: A substance administered or applied to an animal for health or clinical purposes. These include a wide array of pharmaceutical agents such as anesthetics, analgesics, sedatives, anti-infective agents, parenteral fluids, hormones, etc., as well as some non-pharmaceutical substances such as topical cleansers/disinfectants, topical ultrasound transmission substances, styptic agents, etc.

Experimental Substance: A non-therapeutic substance administered or applied to an animal for research or education.

Amendments that can be administratively processed via VVC

The following amendments to an IACUC-reviewed and -approved protocol may be processed administratively by VVC and without full committee (FCR) or designated member review (DMR):

1. Changes to therapeutic substances to include a change in agent, dose, dose interval, or route of administration consistent with the references listed herein, in PubMed, or CAB peer reviewed literature, and/or as recommended by the AV.
2. Change to experimental substances, including a change in test compound, dose, or route of administration, if the change does not result in a change in study objectives or result in greater pain, distress, or degree of invasiveness and is consistent with the references in PubMed, CAB peer-reviewed literature, and/or as recommended by the AV.

*Note that a change from a pharmaceutical grade to a non-pharmaceutical grade drug requires additional justification and will *not* be authorized under this mechanism.

3. Changes in euthanasia to any method approved in the most current AVMA Guidelines for the Euthanasia of Animals.
4. Changes in duration, frequency, type, or number of pre-approved procedures performed on an animal if the change does not result in greater pain, distress, or degree of invasiveness. The AV may authorize minor procedural changes to previously approved protocols providing, in the judgment of the AV, the change will not unduly impact animal welfare (i.e., lessens or involves equivalent pain, acute or chronic stress, distress or effects upon animal welfare) and is consistent with current standards of veterinary practice or specifically addressed in IACUC policy. This includes the following:
 - a. Change in route of blood collection if at least one study staff member has been appropriately trained in the technique (mice and rats only)
 - b. Change in the frequency or volume of blood collected by an approved method, not to exceed more than 1% of the animal's body weight in one collection or over a 24-hour period and no more than 1.5% of the animal's body weight in two weeks (14 days)
 - c. Change in non-invasive procedures for collection of biological samples such as urine or feces, or for collection of anatomic or physiologic data including diagnostic imaging (e.g., Radiography, ultrasonography, and echocardiography), blood pressure measurements, and similar procedures.

*Note: Procedures that require new or revised approvals from a safety committee will not be reviewed by VVC.

- d. Change in method of identification to a method described in the IACUC approved Guidelines if at least one study staff member has been appropriately trained in the method (mice and rats only). Toe clips are excluded from VVC.
- e. Altering behavioral testing methods, providing they do not involve unrelieved pain or distress, do not increase invasiveness, produce different alterations in physiology other than what is already approved, alter expected outcomes, or require additional staff training.
- f. Additional or enhanced enrichment
- g. Changes in stock, strain, or genetic modification, unless the new stock, strain, or modification results in abnormalities that require special support.
- h. Change in breeding schemes so long as the potential for overcrowding is not present.

VVC Process

All changes to a protocol must be submitted to the IACUC via the amendment process for review.

The amendment will receive an administrative review to determine if it meets criteria for VVC and to verify that compliance with the VVC policy is appropriate for the animals in this circumstance. The AV will then confirm whether it qualifies and review the submission. The AV may refer *any* VVC request to the IACUC for review for any reason. Consultation with the AV will be documented, and the PI will receive written confirmation that the change has been reviewed and approved. If the change does not qualify for VVC as determined by administrative or AV review, it will then be sent through the default review route.

A list of protocols administratively reviewed and amended by VVC will be provided to the IACUC at the next scheduled IACUC meeting.

IACUC Approved Reference Documents

Guidance documents that are covered under this policy include the following:

1. Drug formularies are guidance documents listing acceptable uses, dosages, and routes of administration of a wide variety of drugs that may be administered to animals. The following have been approved as a reference by the IACUC:
 - a. ACLAM e-Formulary. Apple iOS App. Version 1.0 (2016) or later. Elsevier, Inc. Carpenter, James W.
 - b. Exotic Animal Formulary. Saunders. Danneman, PJ, Suckow, MA, and Brayton, CF. 2012. The Laboratory Mouse. CRC Press, Boca Raton FL
 - c. Hawk, CT, Leary, S, Morris, TH. Formulary for Laboratory Animals. John Wiley & Sons, Inc.
 - d. Plumb, DC. Plumb's Veterinary Drug Handbook. Wiley Blackwell.
 - e. Peer reviewed journal articles
2. The VVC process may be used to amend the dose, route, concentration, volume, and/or duration of an approved anesthetic, analgesics, or sedative. The following references have been approved by the IACUC:
 - a. Flecknell, Paul. 2009 Laboratory Animal Anaesthesia Third Edition. Comparative Biology Centre, Medical School, Newcastle University, Newcastle UK: Academic Press Gaertner, DJ, Hankenson, FC, Hallman, T, and Batchelder, MA. 2008. Chapter 10.
 - b. Anesthesia and Analgesia in Rodents. In R. E. Fish, M. J. Brown, P. J. Danneman, and A. Z. Karaz (ed.), Anesthesia and Analgesia for Laboratory Animals. Academic Press, San Diego
 - c. Kohn DF, Wixson SK, White WJ, Benson GJ. Anesthesia and Analgesia in Laboratory Animals. Academic Press
 - d. ACLAM e-Formulary. Apple iOS App. Version 1.0 (2016) or later.
3. Other References:
 - a. Animal Welfare Act as amended (7 U.S.C. §§ 2131 et. seq.) Title 9, Code of Federal Regulations, Subchapter A, Section 2.31
 - b. National Institutes of Health Notice Number: NOT-OD-14-126
 - c. National Research Council (2011). Guide for the Care and Use of Laboratory Animals. The National Academies Press: Washington, D.C.
 - d. Public Health Service Policy on the Humane Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare (OLAW). 1985. Rev. 2015.

IMAGE AND VIDEO CAPTURE

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

To comply with regulatory requirements, the ACHE Institutional Animal Care and Use Committee (IACUC) has in effect the following policy which applies to all individuals involved in nonhuman animal research conducted at ACHE RIHWC.

Purpose

The purpose of this guideline is to describe under what conditions photographing, video recording, and audio recording are permitted within the Biomedical Resource Center and in research and teaching laboratories. This guideline has been written to reduce the risk of personnel, students, or guests releasing images or other information related to animal studies at ACHE that could be used inappropriately or interpreted out of context thus endangering the animal care program or the safety of personnel or students working with animals in the research setting.

Definitions:

Biomedical Resource Center – this includes any animal housing facilities on all campuses. Investigator Laboratory – Includes any and all areas outside of the BRC animal facilities where any researcher may perform animal studies.

Photographic Capture – The capture of still or motion images onto any medium or the transmission of any images to another medium via the internet by any means. This includes devices now available for use or that may be invented in the future including (but not limited to) film cameras, videotape, digital disk, digital cameras, and electronic devices such as personal computers, mobile phones or any other personal digital device.

Animal Image – A macroscopic image of any whole live or dead vertebrate animal, which is or has been used, or is intended for use for research, teaching, testing, experimentation, or any other purpose within the programs and facilities. This definition excludes microscopic images.

Audio Capture- The process of recording sound or audio signals, typically from a microphone or other audio input device and converting into a digital or analog format of storage, editing, and playback.

Video Capture- The process of recording video footage from a camera or video source and converting it into a digital or analog format of storage, editing, and playback.

Guidelines

Only approved ACHE personnel (faculty, staff, and students) are permitted to capture images, videos, and sound recordings of research animals.

Image capturing within any animal housing or procedural area requires prior approval by the BRC. Image capturing of animals in laboratories requires the prior approval of the Principal Investigator (PI) as well as the BRC. All/any request(s) for captures images by an outside entity (e.g., news media, vendor) requires prior approval by the Marketing and Communications department and either the Attending Veterinarian or IACUC Chair.

Permission is granted only if:

1. The activity does not interfere with the educational, research or other program functions or events of the Institution.
2. It does not pose a security or safety risk to those individuals involved in the image capture process or to any other persons.
3. It does not cause harm or damage to any ACHE asset.
4. The use or re-use of the resulting image is consistent with the interests of ACHE and other applicable policies.
5. If continuous video or audio recording is conducted in a shared space, permission must be granted by all other PIs who use the space.

Captured Images of Live Animals should meet the following criteria:

1. Appropriate handling and restraining methods for the species must be used and the procedures depicted must be described in an approved IACUC protocol.
2. Animals must have tidy surroundings, clean cages, and clean accessories in all photographs and videos. Water bottles and feeders must be full.
3. All personnel shown in photographs or videos must wear appropriate personal protective equipment (PPE).
4. No references to ACHE or personal identifying information may be visible in photographs or videos, including cage cards, room numbers, ID cards, investigator/staff names, etc.

Captured Images of Deceased Animals should meet the following criteria:

1. The animal must be placed on a clean drape and the area surrounding the animal must be free of any bodily fluids.
2. Images of abnormalities/lesions must be framed and/or cropped to direct attention to that specific area of the animal only.
3. Extraneous, personal, or otherwise sensitive information must not appear in the image or video.

Posting Photographs, Video Recording, and Audio Recording on social media is prohibited unless permission is granted by both Marketing and Communications and either the Attending Veterinarian or the IACUC Chair.

Security Requirements

1. Recordings must not be streamed or saved to personal hand-held devices.
2. Photographs, videos, and audio recordings of research animals cannot be posted to any type of social media, including laboratory websites, unless permitted by Marketing and Communications and either the Attending Veterinarian or the IACUC Chair.
3. All removable storage devices (tapes, flash drives, etc.) that contain photographs, videos, and/or sound recordings of animals must be encrypted and kept in a secure,

locked area with limited access. It is recommended that portable devices include two-factor authentication.

4. Computers or cloud storage containing photographs, videos, and/or audio recordings must be password protected. Copies cannot be made without explicit permission from the PI.
5. All photographs, videos, and/or audio recordings (including copies) must be securely destroyed when they are no longer required for research. Destruction must be consistent with journal, sponsor, and AAALAC requirements.
6. All reasonable attempts to secure smart phones and tablets (e.g., by use of protected passwords, etc.) should be utilized.

Exceptions

1. External inspectors with a regulatory need to visit or inspect animal care activities, such as OLAW, USDA, AAALAC, and certain research sponsors are permitted to capture photographs, videos, and/or audio recording as needed.
2. If an investigator is taking photographs, videos, or audio recordings for publications that are peer reviewed, that peer review will be sufficient for approval.
3. The veterinarian, back-up veterinarian, and BRC staff have ongoing IACUC approval to take photos or videos of animals when needed to assess health concerns.

Procedure

The standard procedure of obtaining permission to capture images within the animal housing facilities and PI laboratories is to fill out a Photo/Video Request Approval form located on the myACHE SharePoint site entitled BRC/IACUC. One form may serve for all pictures and videos taken such that a separate form is not needed for each instance. Verbiage on intent to capture images will also be included in the IACUC protocol form.

Warnings, Responsibilities, and Violations

Unauthorized Photography, Video Recording, and Audio Recording

Use of cameras, cell phones, and other mobile devices capable of capturing images/recordings of animals or animal facilities for personal use is strictly prohibited. Anyone found taking unauthorized photographs or recordings will be immediately removed from the facility and must relinquish any images or recordings obtained without permission.

Responsibilities

All individuals to whom this guideline applies are responsible for becoming familiar with and following this guideline. University supervisors are responsible for promoting and understanding this guideline and for taking appropriate steps to ensure compliance with it.

Reporting a Violation

All are encouraged to report violations of this guideline anonymously through the animal welfare reporting hotline by calling: **479-434-4050**.

ANIMAL ADOPTION POLICY

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

Animals that have concluded studies may be made available for adoption/acquisition by individuals. The animals must be healthy at the time of adoption/acquisition as determined by the Veterinarian. The animals must not have had any type of chemical/biological treatments or surgical procedures performed on them prior to the adoption/acquisition. The animals must not be transgenic or immunodeficient. Animals must be altered prior to adoption (with the exception of mice and fish).

Individual requests should be made to the Veterinarian. Requests must come from individuals associated with ACHE who have completed animal care training for the species being adopted. Animals may only be adopted to serve as companion animals. The appropriate forms must be completed and reviewed by the IACUC prior to the adoption/acquisition.

BLOOD COLLECTION POLICY

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

Scope

This document provides information on blood collection methods for common laboratory animals. All procedures must be approved by the Institutional Animal Care and Use Committee (IACUC). The method of blood collection to be used, the intervals between blood collection procedures, and the volume of blood to be removed must be listed in the approved protocol specific to each study. Contact the BRC if training is needed for specific blood collection methods and techniques.

Procedures

The following criteria are provided to determine the maximum safe amount of blood to be withdrawn. It is recommended that no more blood is withdrawn than is necessary. Remember to calculate beforehand the minimum amount of blood necessary to perform all tests and assays, and that the serum fraction is about $\frac{1}{2}$ of the total blood volume. When calculating blood volumes based on body weights (see below), remember that body weights in kilograms (kg) will convert to blood volumes in liters, and weights in grams (g) will convert to volumes in milliliters (ml).

Approximate Blood Volume:

- 5-10% of body weight= total blood volume
 - The circulating blood volume for rodents can generally be estimated as 55-70 ml/kg of total body weight. However, care should be taken in these calculations as the % of total blood volume will be lower (-15%) in obese and older animals.
 - See table at end of this document for some specific blood volumes.

Blood Collection Volumes:

- 1% of body weight is the maximum volume withdrawn over 14 days, without requiring supplemental fluids. This applies for total blood collections as well as repeated collections. For irregular sampling schedules, calculate the total amount needed over a 14 day span
- 0.07% of body weight is the amount that can be taken daily without requiring supplemental fluids. • 4-5% of body weight would result in exsanguination

Single Blood Draw:

Maximum of 1% of body weight can be removed as a single blood draw every 14 days, without requiring administration of supplemental replacement fluids. Withdrawing the minimum amount of blood necessary is strongly recommended.

Examples:

- 0.15 ml from a 15 g mouse
- 3 ml from a 300 g rat
- 30 ml from a 3 kg rabbit

Multiple Blood Draws:

If the total volume withdrawn over a 14-day period is less than or equal to 1% BW, then no additional action needs to be taken. If the total volume withdrawn over a 14-day period is up to >1%/up to 2% BW, fluid volume replacement must be considered. Withdrawing the minimum amount of blood necessary is strongly recommended.

Examples:

- Up to 0.15 ml withdrawn from a 15 g mouse over 2 weeks is OK
- Up to 0.3 ml withdrawn from a 15 g mouse over 2 weeks, replace volume with 0.3 ml saline SC
- Up to 3 ml from a 300 g rat over 2 weeks is OK
- Up to 6 ml from a 300 g rat over 2 weeks, replace volume with 6 ml saline SC

Exsanguination:

Approximately 50-75% of total blood volume (4-5% BW) can be obtained by terminal exsanguination. The animal must be deeply anesthetized, or recently euthanized, prior to exsanguination. Since the amount of blood obtained is substantially increased if the heart is beating during the bleeding procedure, use of a surgical plane of anesthesia is required. The procedure for anesthesia and/or euthanasia must be described fully in the approved IACUC protocol.

Fluid Replacement:

If the volume of blood removed from an animal exceeds the maximum recommended blood collection volumes (i.e. >1% BW every 14 days), replacement of the removed volume of blood with warm (30-35°C) isotonic solution (e.g. 0.9% saline (normal, physiologic), lactated Ringer's solution) constitutes accepted veterinary practice. When this volume of blood is harvested, it should be withdrawn at a slow, steady rate, and the volume of solution to be infused should be administered similarly.

Monitoring:

If too much blood is withdrawn too rapidly or too frequently without replacement (approximately 2% of the animal's body weight at one time), the animal may experience hypovolemic shock. If signs of shock are observed, contact BRC staff immediately. Stressed, sick, or otherwise compromised animals may not tolerate the blood collection criteria noted above, which is for healthy animals. By monitoring species appropriate hematocrit (HCT or PCV) and/or hemoglobin (Hb) it is possible to evaluate if the animal has sufficiently recovered from single or

multiple blood draws. Remember it may take up to 24 hours for hematocrit or hemoglobin to reflect a sudden or acute blood loss.

Blood Collection Sites and Methods:

The IACUC recommends labs reference the NC3R website to learn about the different methods of blood withdrawal and the volumes you will be able to obtain from each method. This website also provides a decision tree to decide which method is best for your study.

Table 2 Below is a comprehensive table of total blood volumes and blood sample volume limits that are considered generally safe for healthy laboratory animals, domestic species and non-human primates (adapted from Wolfensohn S and Lloyd M 2003 [1]): [Blood sampling: General principles | NC3Rs](#) -

Species	Reference Weight (g)	Blood Volume (ml/kg)\$	Total Blood Volume (TBV), Normal Adult (ml)	Safe Volume for Single Bleed (ml)*	Bleed Out Volume (ml)
Mouse	18-40	58.5	Male: 1.5-2.4 Female: 1.0-2.4	0.1-0.2	Male: 0.8-1.4 Female: 0.6-1.4
Rat	250-500	54-70	Male:29-33 Female: 16-19	Male: 2.9-3.3 Female: 1.6-1.9	Male: 13-15 Female: 7.5-9
Hamster	85-150	78	Male: 6.3-9.7 Female: 7.1-11.2	Male: 0.6-0.9 Female: 0.7-1.1	Male: 2.9-4.5 Female: 3.3-5.2
Gerbil	55-100	66-78	Male: 4.5-7 Female: 3.8-6	Male: 0.4-0.7 Female: 0.4-0.6	Male: 2.2-3.5 Female: 1.9-2.9
Guinea Pig	70-1200	69-75	Male: 59-84 Female: 48-63	Male: 6-8 Female: 5-6	Male: 29-42 Female: 24-31
Rabbit	1000-6000	57-65	58.5-585	5-50	31-310
Ferret	600-2000	70	42-140	4-14	21-70
Dog	-	70-110#	900-1170a	90-110	-
Cat	-	47-65	140-200	14-20	-
Pig- Large White	-	56-69	13,200-15,000	1320-1500	-
Pig- Yucatan	-	56-69	4200-4800	420-480	-
Sheep	-	58-64	4060-4480	400-500	-
Goat	-	57-90	3990-6300	400-630	-
Cattle	-	60	27,000-36,000b	2700-3600	-
Horse	-	75	33,750-45,000b	3375-4500	-
Marmoset	-	60-70	21-24.5	2.1-2.4	-

Rhesus macaque	-	55-80	Male: 420-770 Female: 280-630	Male: 42-77 Female: 28-63	-
Long-tailed macaque	-	50-96	Male: 280-560 Female: 140-420	Male: 28-56 Female: 14-42	-

\$ A blood volume estimate for a single species may not reflect differences among individual breeds or variations due to age, size, or illness

** Single bleed of 10% total blood volume*

Much breed variation

a Beagle

b Assumes adult weight 450-600 kg

POPULATION DENSITY-BREEDING MICE

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

This policy identifies the numbers of breeding mice which can be housed in polycarbonate cages of at least 75 sq. inches of floor space. These guidelines are based upon housing recommendations from the 8th edition of “The Guide for the Care and Use of Laboratory Animals”, and on best management practices to support research, prevent overcrowded conditions, and assure litters are weaned appropriately.

75 sq. inch caging may house:

- Trio breeding schemes of one (1) adult male and two (2) adult females.
 - Acceptable for inbred, transgenic, or other strains of mice that produce smaller litters.
 - A maximum of 9 pups in any cage with the 3 adults. In cages found to exceed 12 pups, one of the females with her litter will be removed from the cage. It is best practice to remove the females when they are obviously pregnant.
 - Pups weaned at 21 days*
- Trio breeding schemes of one (1) adult male and two (2) adult females for outbred, hybrid, and strains that produce larger litters, only if
 - One pregnant female is removed from the cage prior to parturition.
 - There is only one litter per cage with up to 2 adults.
 - Pups are weaned at 21 days*
- Monogamous pairing of one (1) adult male and one (1) adult female for strains with larger litters.
 - Pups are weaned at 21 days*

In the case of either monogamous breeding or trio breeding as described above, older litters must be weaned if female is bred at postpartum estrus and has a second litter. The presence of the older litter can cause the parents to cannibalize the newborn pups or be trampled by so many older mice in the cage.

It is the responsibility of the Principal Investigator to record accurate birth dates on cage cards for calculating weaning dates. Exceptions to these guidelines are an exception to “The Guide” and will require IACUC approval.

Compliance with IACUC Guidelines on Cage Population Densities for Breeding Mice at ACHE:

1. BRC staff will flag cages they find do not comply with the above policy.
2. BRC staff will inform the BRC Manager or Veterinarian that cages were flagged.
3. The BRC Manager or Veterinarian inform the PI of the situation, and The PI will be given 24 hours to correct the situation.

4. If the situation is not corrected within 24 hours, the BRC staff will alleviate crowded cages and charge for tech time.
5. If the BRC has recognized a PI is a recurrent offender (two or more occurrences), the matter will be brought to the attention of the IACUC.

Weaning pups:

1. Male and female pups are separated at weaning into separate cages with NO more than 5 mice per cage.
2. If a litter contains a single animal of one sex then place a gel cup in the cage with the pup and extra enrichment.
3. Place a few pieces of rodent feed on the floor of the cage with ALL newly weaned pups in addition to filling the hopper with rodent feed and providing a clean water bottle.

Again, you must have IACUC approval to extend the weaning age past 21 days. If a second litter is born, the older litter must be weaned even if IACUC approval for extended weaning age has been given.

SIGNIFICANT VS. MINOR CHANGES TO IACUC PROTOCOLS

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

To comply with regulatory requirements, the ACHE Institutional Animal Care and Use Committee (IACUC) has in effect the following guideline.

The PHS Policy on Humane Care and Use of Laboratory Animals (Policy) ([IV.C.1.](#)) and Animal Welfare Regulations ([9 CFR 2.31 \(d\) \(1\) \(i\)- \(iv\)](#)) define the responsibilities of the IACUC regarding review and approval of proposed significant changes to animal activities. Changes to approved research projects must be conducted in accordance with the institution's Assurance, the US Department of Agriculture (USDA) Animal Welfare Act and Animal Welfare Regulations, and must be consistent with the [Guide](#) unless an acceptable justification for a departure is presented. Additionally, IACUCs are responsible for assuring that the changes to approved animal activities meet the requirements described in the PHS Policy [IV.C.1.a-g](#).

All proposed changes to an approved animal care and use protocol must be approved by the IACUC before they are instituted.

Determination of Modifications

The IACUC has established the following guidelines to determine whether a requested modification is a minor or significant change to an active IACUC approved protocol. Contact the Consulting Veterinarian or the IACUC Chair for guidance if in doubt whether a proposed change is "minor" or is "significant".

The following categorization of activities is not exhaustive, but it intended as a guide to the type of changes that the IACUC might regard as major or minor changes to a protocol.

Minor Modifications

A minor modification is a change to the approved protocol activity that is unlikely to be a physiological cost to the animal, or the change may decrease the potential for pain/distress (i.e. a refinement).

1. Reduction in the proposed number of animals
2. Transfer of animals to another protocol where animals (same stock/strain) are already approved on that study
3. Addition or removal of animal vendors approved by the BRC Facility Manager and/or Veterinarian.
4. Addition or removal of personnel, other than the Principal Investigator, or personnel performing surgery and/or euthanasia (see Summary of Personnel Modifications to Protocols below).
5. Changes in title or funding agency with the animal protocol being the same

6. Adding or changing the location where animal procedures are conducted if locations have been inspected by the IACUC.
7. One time addition of animals Changes of less than 10% or no more than 5 animals of mice of the genus, *Mus* and rats of the genus *Rattus* that are bred for use in research only may, at the IACUC's discretion, be considered minor

Major Modifications/Significant Changes

A significant modification is defined as any change to the approved protocol activity that is likely to be a physiological cost to the animal (i.e. there is a potential to increase pain/distress).

1. Change in the study objectives and goals.
2. Any procedure that has the potential to increase pain/distress.
3. New or additional procedures, especially those that involve more than momentary restraint, or other surgical or invasive procedures not listed in the approved protocol activity.
4. When housing or using animals in a location that is not a part of the animal care and use program that is overseen by the IACUC.
5. Any change in procedure that would impact personnel safety.
6. Use of hazardous agents in the animal procedures (this could mean chemical, biological or test compounds).
7. Changes in the dose/volume of experimental materials , changes in dose route, or dose frequency that result in a change in study objectives or result in greater pain, distress, or degree of invasiveness and are not consistent with the references in PubMed, CAB peer-reviewed literature, and/or as recommended by the AV.
8. Food/water restriction (not routine fasting).
9. Proposals to switch from non-survival to survival surgery.
10. Changes in species.
11. Change in the number of animals needed over the original number approved (more than or equal to 10%)
12. Withholding of analgesics.
13. Changes in euthanasia or methods of euthanasia that are not AVMA approved
14. Extensions of the study or changes in the PI.
15. Change in the duration, frequency, or number of procedures performed on an animal resulting in greater pain, distress, or degree of invasiveness
16. Change in age of animals ordered or used.
17. Change that would require animals to be fed, housed or cared for in any way that is not standard for that species, or does not meet that species' minimum requirements as set by the Guide

Changes that May Be Handled Administratively Without IACUC Approval

1. Correction of typographical errors
2. Correction of grammar
3. Correction of training completion status
4. Contact information updates
5. Addition or deletion of personnel who do not perform surgery nor euthanasia and have completed all components of the ACHE Animal Care and Use Training (including OHS training and documentation).

Summary of Personnel Modifications to Protocols

1. New personnel will be added and be approved by the IACUC Coordinator through an administrative modification when:
 - a. The personnel are not performing euthanasia nor surgical procedures on animals and;
 - b. The personnel have completed all components of the ACHE Animal Care and Use Training (including OHS training and documentation).
2. New personnel can be approved by the DMR method (unless called to FCR) when:
 - a. The personnel are performing euthanasia and/or surgical procedures on animals and;
 - b. The personnel have completed all components of the ACHE Animal Care and Use Training (including OHS training and documentation).
 - c. The personnel have completed appropriate euthanasia and/or surgical training.

PEST CONTROL

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

Background

The "Guide for the Care and Use of Laboratory Animals" (Guide), NRC 2011 indicates that pest control managed by the animal care and use program personnel should be designed to prevent, control, or eliminate the presence of or infestation by pests in the animal environment. The *Guide* states (pg. 74) that "If traps are used, methods should be humane; traps that catch pests alive require frequent observation and humane euthanasia after capture" and also notes (pg. 112) that "all animals should be observed for signs of illness, injury or abnormal behavior by a person trained to recognize such signs. As a rule, such observation should occur at least daily." The *Guide* (pg. 105) further indicates that an adequate veterinary care program consists of assessment of animal well-being and effective management of pain and distress. The Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research, an AAALAC International Reference Resource, indicate that lethal traps should result in a clean, effective kill and should be checked at least once a day, and if an animal is still alive, it should be immediately dispatched in accordance with guidelines of the American Veterinary Medical Association.

Arthropod pest control is contracted with an outside company on an as needed basis.

Procedures

Pest control

1. Insect Pest Control:
 - a. Any indication of insect infestations should also be reported to the BRC Manager or the Attending Veterinarian immediately.
 - b. Treatment of insect infestation is contracted with facilities or an outside company as needed.
2. Rodent Pest Control:
 - a. The BRC facility has live rodent traps in every animal holding room, the necropsy room, as well as food/water/bedding storage areas.
 - b. Food will be placed in each live trap along with the date the food is placed. The food should be replaced once every 6 months or more often if needed.
 - c. The traps are checked daily, and all findings are recorded on the daily check sheets.
 - d. Any animals found in or around the rodent bait stations should be euthanized immediately (if still alive). The veterinarian will be notified so the animal can be tested for diseases that may affect research animals.

- i) Caution should be taken when handling any wild animals that may be caught. Gloves and thorough hand washing are recommended.

PUBLIC ACCESS TO ANIMAL FACILITIES

Approved: 1/15/25

Last Reviewed: 1/15/25

Revision History:

The following individuals have unrestricted access to ACHE animal facilities:

- IACUC Chair
- Institutional Official
- Biomedical Resource Center Staff
- ACHE Principal Investigators with IACUC approved protocols
- PI staff working on IACUC approved protocols who have received the required training
- Pre-approved Facilities Services Staff

All tour requests must be made to the BRC Manager or Veterinarian. The request must state the reason for requesting access. Public Access will be granted for informational purposes only.

The Animal Care Manager, Veterinarian, and Principal Investigator(s) must approve of the time of the public access.

All public access will be guided by an appropriate ACHE Representative as determined by the BRC Manager and/or the veterinarian.

Guests are not allowed to handle animals. Guests are not allowed to enter barrier rooms (e.g. quarantine rooms, SPF rooms.) or ABSL2 rooms. Guests should only have access to rooms/labs that will not cause any disruption to the animals' wellbeing or the research taking place as determined by BRC staff and PI.

If guests have been in another facility within 48 hours and need to enter ACHE animal facilities, they should consult with the veterinarian or BRC manager (access may be denied in such an instance).

Unauthorized electronic devices (including but not limited to, recording devices such as tape recorders, video recorders, cameras, cell phones, etc.) are not allowed and/or must be turned off prior to entering the facility. Guests must get prior approval to use them.

Guests under the age of 18 will not be permitted public access without adequate adult supervision. A Parent must sign the guest form.

The number of people allowed public access at one time will be restricted based on the size of the area to be toured and the nature of the visit.

All animal facility guests must adhere to the Personal Protective Equipment Procedures. The veterinarian or BRC Manager may determine that additional Personal Protective Equipment is necessary.

All guests will be required to review and sign the “Occupational Health Program for Guests Requesting Access to the Biomedical Resource Center”.

ESCAPED RODENT GUIDELINES

Approved: 3/12/25

Last Reviewed: 3/12/25

Revision History:

Purpose:

This guideline outlines the steps to take if a rodent escapes and the individuals/authorities that need to be informed.

Guideline:

Rodents that come into contact with the floor or unintended areas need to be placed in a separate cage and placed into quarantine for three months to avoid contamination of other rodents. At the end of the three months, a PCR panel will be submitted and the rodent will only be allowed to return to the colony if it is free of excluded pathogens. Alternatively, the animal may be euthanized.

Rodents that escape need to be reported to the proper personnel (listed below) to aid in recovery and any hazard reporting.

Procedure:

If a rodent escapes from the cage/housing area, encounters the floor or unintended areas, and is retrieved: place the animal in a single cage; note home cage location, if known, on new cage card; document on home cage card that a rodent escaped and was moved; and notify the BRC manager and PI via email. If the rodent is a USDA covered species the Chair of the IACUC must also be notified.

NOTE: If a rodent escapes and is not found, **immediate action is required**. Call the BRC Manager and the PI immediately.

If the escaped rodent (found or not found) is transgenic or has been treated with biologicals, include biosafety on the notification. Report the following information: PI on study, if the animal is transgenic, what biological material was administered to the rodent, if rodent is male or female, was it found, or is it still unaccounted for (if found, where the animal was found).

If the rodent has been treated with chemicals (found or not found), include chemical safety on the notification.

If the escaped rodent (found or not found) has been treated with radiation, include radiation safety on the notification.

If the rodent is not found, a live trap, obtained from the vivarium, should be placed in the area where the rodent was last seen. Ensure the trap is baited with food. When set, live traps must be checked daily. Personal Protective Equipment (PPE) should be worn appropriately when

checking the trap. If a wild rodent is inadvertently trapped contact the BRC manager. If the rodent is located, report it to the same individuals the missing rodent was initially reported to.

GLOSSARY

ALIQOT a portion of a larger whole, especially a sample taken for chemical analysis or other treatment.

ALTRICIAL born helpless and requiring significant parental care.

ANALGESIA AGENT any member of the group of drugs used for pain management.

ANIMAL IMAGE a macroscopic image of any whole live or dead vertebrate animal, which is or has been used, or is intended for use for research, teaching, testing, experimentation, or any other purpose within the programs or facilities. This definition excludes microscopic images.

ASCITES a condition where excess fluid accumulates in the abdominal cavity. The fluid can cause swelling, discomfort, and other complications.

AUDIO CAPTURE the process of recording video footage from a camera or video source and converting it into a digital or analog format of storage, editing, and playback.

BIOMEDICAL RESOURCE CENTER this includes any animal housing facilities on all campuses.

BUCCAL SWAB a painless procedure that involves collecting cells from the inside of the cheek.

CACHEXIA a condition that causes significant weight loss and muscle loss.

CATABOLISM the breakdown of complex molecules in living organisms to form simpler ones, together with the release of energy; destructive metabolism.

CONFIDENTIAL BUSINESS INFORMATION commercial or financial information considered to be confidential because disclosure may: (1) Impair the Government's ability to obtain necessary information in the future; or (2) Cause substantial harm to the competitive position of the individual or business entity who provides the information.

CONTROLLED SUBSTANCE any drug, substance or analog compound that falls under any of the five (I-V) schedules identified by the United States Department of Justice/Drug Enforcement Administration and as defined by the Controlled Substance Act (CSA).

CYANOSIS a bluish-purple discoloration of the skin and mucous membranes caused by low levels of oxygen in the blood.

DRUG any regulatory agency approved or investigational substance, agent, biologic, or chemical listed in a pharmacopeia, chemical supply catalogue, synthesized or isolated in a laboratory, and administered to animals by any route, including injection inhalation, topical application for use in the investigation, diagnosis, cure, mitigation, treatment or prevention of disease or biology in humans or animals.

DYSPNEA shortness of breath or difficulty breathing.

EUTHANASIA the humane disposition of an animal accomplished by a method that minimizes or eliminates pain and distress. This is routinely accomplished by rapid unconsciousness and subsequent death.

EXOPHTHALMOS a medical condition where the eyeballs protrude or bulge out of their sockets.

EXSANGUINATION the loss of blood from the circulatory system of a vertebrate, usually leading to death.

EXUDATE a fluid that leaks out of blood vessels into surrounding tissues.

FOMITES inanimate objects that can carry and spread disease and infectious agents.

FOOD REGULATION scheduled access to food or fluid sources during which an animal may consume as much as desired at regular intervals.

FOOD RESTRICTION the provision of rations such that the volume of food or fluid is strictly monitored and controlled.

FRY young fish that have recently hatched and are able to feed on their own.

HEMOSTASIS a physiological process that prevents excessive blood loss after an injury.

ICTERUS technical term for jaundice.

INVESTIGATOR LABORATORY includes any and all areas outside of the BRC animal facilities where any researcher may perform animal studies.

LACRIMATION shedding tears, or shedding more tears than is normal.

MACERATION use of a specially designed mechanical apparatus having rotating blades or projections, causing immediate fragmentation.

MALOCCLUSION a misalignment of the teeth and jaws, resulting in an improper bite.

MANIPULANDA nylabones, wooden, or other chew toys used for gnawing are the standard enrichment for rats.

MEDICAL MATERIAL a non-bioactive substance intended to be administered to animals for use in the diagnosis, cure, mitigation, treatment, or prevention of disease, or a substance intended to affect the structure or function of the body (e.g., catheters, sutures). Food is not considered a medical material.

MICROPHTHALMIA a rare eye condition that causes one or both eyes to be abnormally small.

NECROPSY a post-mortem examination of a non-human animal.

NEONATE a newborn child (or other mammal) less than four weeks old. In this instance, mouse or rat pups up to 10 days of age.

NEOPLASIA a process in which new, abnormal tissue growth occurs.

OPERCULAR relating to or denoting a structure that closes or covers an aperture such as a fish's gills.

PARAPHIMOSIS a medical condition where the foreskin becomes trapped behind the glans penis, cutting off blood supply.

PARESIS a partial loss of muscle strength or voluntary movement.

PARTURITION the action of giving birth to young; childbirth.

PHARMACEUTICAL GRADE COMPOUND any active or inactive drug, biologic, reagent, etc. which is approved by the FDA or for which a chemical purity standard has been written/established by any recognized pharmacopeia (book/compendia) such as: US Pharmacopeia (USP), National Formulary, British Pharmacopeia.

PHOTOGRAPHIC CAPTURE the capture of still or motion images onto any medium or the transmission of any images to another medium via the internet by any means. This includes devices now available for use or that may be invented in the future including (but not limited to) film cameras, videotape, digital disk, digital cameras, and electronic devices such as personal computers, mobile phones or any other personal digital device.

PHYSICAL RESTRAINT according to (The Guide, 2011), “the use of manual or mechanical means to limit some or all of an animal’s normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation.”

PRECOCIAL able to independently feed and move almost immediately at birth.

PROPRIETARY INFORMATION information or data belonging to an owner or proprietor, who may have exclusive rights to the manufacture and sale of a specific item.

PTOSIS a condition where the upper eyelid droops, covering part of all of the pupil.

RALES abnormal lung sounds characterized by clicking, bubbling, or rattling noises heard during inhalation.

RAT TICKLING a technique that involves gently wrestling a rat with your hand to mimic play to improve rat welfare and fear. The standard method is to tickle the back of the rat’s neck, the flip it over and tickle its underside.

REGISTRANT a registered person with the DEA and/or Arkansas Board of Pharmacy to use Controlled Substances.

REGISTRATION the permit obtained from the DEA and/or the Arkansas Board of Pharmacy to use Controlled Substances.

RESEARCH any scientific investigation.

SENESCENT aging or growing old.

SENSITIVE INFORMATION information or data in which disclosure, loss, misuse, alteration, or destruction may adversely affect national security or other government (usually Federal) interest.

SKIN TURGOR the elasticity and resiliency of the skin. Indicates the body’s hydration status.

STYPTIC AGENT a substance that stops bleeding by constricting blood vessels.

SUSPENSION a temporary halt in any ongoing research activities, including collecting new animals or activities involving previously collected animals.

TACHYPNEA abnormally rapid breathing rate.

TERMINATION a permanent halt in all ongoing research activities and closure of the project.

TRADE SECRET any formula, pattern, device or information that is used in business which provides a competitive advantage.

TUNNEL HANDLING a non-aversive method of picking up mice by guiding them into a tube instead of directly handling them.

ZOONOSIS an infectious disease that can be transmitted from animals to humans caused by a pathogen, such as a virus, bacterium, parasite or fungus.