

# LIBERTY UNIVERSITY

INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

## IACUC Policies & Guidelines Handbook

### Contents

Animal Care and Use .....	3
Guidelines .....	3
Anesthesia and Analgesia in Laboratory Animals .....	3
Blood Sample Withdrawal .....	5
Counting the Number of Animals on a Protocol .....	7
Handling, Preparing, and Using MS-222 .....	8
Humane Endpoints .....	10
Injections in Laboratory Animals .....	11
Recordkeeping and Perioperative Care and Monitoring .....	15
Reporting Adverse Events .....	17
Restraint in Animal Teaching and Research .....	18
USDA Pain and Distress Categories in IACUC Protocols .....	20
Use of Expired Products .....	24
Veterinary Drug Labeling .....	25
Policies .....	26
Animal Escape and Notification .....	26
Avian Embryo Use .....	28
Breeding of Rodents .....	29
Food or Water Restriction .....	32
Isolation of Sick Animals .....	33
Survival Surgeries .....	34
Use of Zebrafish .....	36
Animal Disposition .....	38
Policies .....	38
Euthanasia of Laboratory Mice and Rats .....	38
Protocol Closure or Transfer in the Event of a PI's Absence .....	40
Animal Facilities & Housing .....	41
Guidelines .....	41
Cage Card System .....	41
Tracking Animal Use .....	43
Policies .....	45
Policy Regarding Animal Husbandry, Care, and Documentation .....	45
Animal Identification .....	48
Approval of New Animal Facilities .....	50
Group Housing of Social Species .....	51
Holding of Animals not on Active Research Protocols .....	52
Housing and Environmental Enrichment for Laboratory Animals .....	54
Mice Cage Sizing and Density .....	58
Non-Vivarium Animal Housing .....	60
Quarantine and Stabilization of Research Animals .....	61
Transportation of Research Animals .....	62
Instructional Protocol Specific .....	64
Policies .....	64
Animal Use in Learning or Teaching .....	64

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Miscellaneous .....	66
Policies.....	66
Inter-Institutional Animal Research.....	66
Post Approval Monitoring .....	68
Guidelines .....	68
Congruence for NIH Funded Animal Studies.....	68
Policies.....	70
Non-Compliance .....	70
Reporting of Animal Concerns .....	72
Reporting to External Agencies .....	73
Review of Animal Welfare Concerns .....	74
Protocols .....	76
Guidelines .....	76
Performing a Literature Search for Alternatives.....	76
Policies.....	78
Administrative Review of Protocol Amendments .....	78
Amendments Requiring a New Protocol .....	79
Closure of Active IACUC Protocols by PI Request or Expiration.....	80
Eligibility and Responsibilities of the Principal Investigator .....	81
Exempt Animal Protocols .....	83
Genetically Modified Animals.....	85
IACUC Protocol Submission.....	86
Procurement of Laboratory Animals.....	87
Use of DMR Subsequent to FCR for Protocol Modification Requests .....	88
Use of Literature Searches for Alternatives .....	89
Training & Personnel.....	90
Guidelines .....	90
Health and Safety .....	90
Short Term Researcher Training.....	91
Policies.....	93
Training of Principal Investigators, Researchers, and Animal Care Staff .....	93
Approval and Revision History .....	95

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Animal Care and Use

#### Guidelines

#### Anesthesia and Analgesia in Laboratory Animals

### **GUIDELINES FOR ANESTHESIA AND ANALGESIA IN LABORATORY ANIMALS**

#### **PURPOSE:**

It is federally mandated that any animals undergoing potentially painful teaching or research procedures be provided with proper anesthesia or analgesia to mitigate pain. Researchers and instructors are mandated with mitigating pain and distress in animals used for research and instruction at Liberty University. Exceptions to the following policies are only permitted with proper justification when scientifically necessary and must be approved by the Liberty University IACUC.

- The Office of Laboratory Animal Welfare PHS Policy on Humane Care and Use of Laboratory Animals Principle #4 states, “Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative.”
- USDA Animal Care Policy #11 defines a painful procedure as “any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being.”

#### **GUIDELINES:**

The determination of whether or not a procedure is likely to involve pain or distress to an animal will be assessed during the veterinary protocol review process. An appropriate pain management plan including anesthesia shall be determined on a case-by-case basis. If a protocol includes a component where a specific pain level is necessary to obtain scientifically justifiable data, such justification must be provided in the IACUC protocol and be subsequently approved by the IACUC. For help determining specific USDA pain criteria, see the guidelines on determining pain and distress categories.

#### **TRAINING**

Personnel who are performing anesthesia and/or surgery must be properly trained to do so. Principle Investigators are responsible for ensuring that laboratory or instructional staff are adequately trained and/or certified prior to performing procedures.

#### **ANESTHETICS**

*For the use of any anesthetic agent, the attending veterinarian must be consulted regarding proper dose and administration for the species and specific procedure.*

- Inhalant Anesthetics (Ex: Isoflurane)
- Injectable Anesthetics (Ex: Ketamine Combinations, Dexmedetomidine)
- Immersion Anesthetics (Ex: Buffered MS-222)
- Local Anesthetics (Ex: Lidocaine, Bupivacaine)

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### **ANALGESICS**

*For the use of any analgesic agent, the attending veterinarian must be consulted regarding proper dose and administration for the species and specific procedure.*

- Opioids (Ex: Buprenorphine, Morphine)
- NSAIDS (Ex: Meloxicam, Carprofen, Ketoprofen)

### **MONITORING**

Mammals must never be left alone during anesthesia. To ensure proper depth, anesthesia and analgesia shall be monitored and recorded at least every 15 minutes. Intra and post-operative monitoring plans must be included in the protocol submission.

Other monitoring might be necessary depending on the procedure. Such measures may include heart rate, blood pressure, body temperature, and tissue oxygenation. Monitoring must occur and be recorded from post-operation to complete recovery.

### **RECORD KEEPING**

All administration of analgesia, anesthesia, or peri-operative observation must be properly recorded. Depending on the species, records may be kept in an animal's individual medical record, in laboratory records, or on post-operative cage cards.

### **STATEMENT ON CONTROLLED SUBSTANCES**

Several commonly used anesthetics and analgesics are controlled substances and require certain authorizations and procedures prior to use in animal research. Once obtained, remember that controlled substances must be properly stored with appropriate record keeping. Depending on the agent used, licenses or permits may be required by the State of Virginia. It is the responsibility of the PI to determine if any additional authorization is needed prior to performing any procedures.

*Prior to using any controlled agents, consult the attending veterinarian.*

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Blood Sample Withdrawal

## **GUIDELINES REGARDING BLOOD SAMPLE WITHDRAWAL**

### **PURPOSE:**

The Liberty University IACUC has developed the following guidelines regarding the use of phlebotomy for common laboratory animal species and will provide guidance on recommended collection volumes and frequency. Animal wellness is a primary consideration when blood sampling is conducted, and the animal's physiological response and impact must be monitored.

### **GUIDELINES:**

When submitting a protocol involving blood collection, keep the following in mind:

- When submitting a protocol to the IACUC, indicate that personnel will remain in compliance with this document if blood collection will be performed.
- Any deviation from these standards must be documented, justified, and approved.
- Literature on the history or necessity of the procedure must be provided.
- Provisions must be made for appropriate fluid replacement.
- The PI must ensure that all laboratory personnel are adequately trained and have demonstrated competency in blood collection.
- Ensure that appropriately sized needles are being used for the species listed on the protocol.

**All blood collection procedures must be consistent with current best practices.** Proper methods of blood collection for all species can be confirmed with the Attending Veterinarian.

### **USE OF RESTRAINT**

- For the purposes of blood collection, larger animals such as dogs, cats, cows, horses, and sheep only require physical restraint.
- Small animals, such as rabbits, mice, rats, and other rodents may be placed in appropriate restraint devices.

### **PHLEBOTOMY**

Circulating blood volume (CBV) shall be determined from known species-specific volume to weight values and not calculated on flat percentage of body weight.

**TABLE I. CBV LIMITS AND RECOVERY PERIODS**

<i>Single Sampling</i>		<i>Multiple Sampling</i>	
% circulatory blood volume removed	Approximate recovery period	% circulatory blood volume removed	Approximate recovery period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
15%	4 weeks	20%	4 weeks

**TABLE II. ESTIMATED CBV IN COMMON LAB ANIMAL SPECIES**

Species	Estimated CBV
Dog	85ml/kg

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## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Cat	55ml/kg
Rat	65ml/kg
Mouse	75ml/kg
Guinea Pig	70ml/kg
Hamster	80ml/kg
Rabbit	55ml/kg

# LIBERTY UNIVERSITY

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### Counting the Number of Animals on a Protocol

## **GUIDELINES ON COUNTING THE NUMBER OF ANIMALS ON A PROTOCOL**

### **PURPOSE:**

The Liberty University IACUC has established the following guidelines for counting animals used for teaching, research, or breeding at LU. For the purposes of this document, *animal* shall refer to any non-human vertebrate.

### **GUIDELINES:**

When counting the number of animals on a protocol, use the following guidelines set forth by the Liberty University IACUC:

- The PI must maintain current, accurate records of animal use in his or her approved protocol, including any animals purchased, transferred, or bred for the purposes of the protocol.
- Each animal should only be counted once for the duration of the protocol.
- Animals that must be counted include:
  - Animals purchased or transferred onto the protocol
  - Any animals bred for the protocol (must be counted at first observation of live birth).
    - Animals that are not born “alive” do not need to be counted. If 5 pups are born in a litter, but only 2 are alive, “2” would be the number counted on the protocol.
- An “animal welfare walkthrough” shall be performed on a routine basis by vivarium staff in order to maintain an accurate count of animals.
  - The vivarium staff member conducting the walkthrough will make note of any unusual behavior or animal death, which will then be reported to the responsible PI.

# LIBERTY UNIVERSITY

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### Handling, Preparing, and Using MS-222

## **GUIDELINES ON HANDLING, PREPARING, AND USING MS-222**

### **PURPOSE:**

The anesthetic agent, MS-222, or Tricaine methanesulfonate, is used primarily for the sedation, anesthesia, and euthanasia of fish, amphibians, and other aquatic, ectothermic animals. These guidelines seek to inform users of its proper handling and use.

### **GUIDELINES:**

The sole responsibility of ensuring the safe use of MS-222 lies with the Principal Investigator. All personnel using MS-222 must be properly trained in its handling, use, and preparation.

### **STORAGE**

MS-222 powder (Finquel or Tricaine-S) must be stored at room temperature in a dark cabinet.

### **SAFETY PRECAUTIONS**

Gloves, eye protection, facemasks, and lab coats must be worn in order to avoid skin contact when preparing MS-222 solutions from a powder. If MS-222 is being used in the field, utilize proper precautions including but not limited to gloves and eye protection.

### **PREPARATION**

MS-222 solutions must be prepared in a well ventilated area, under a functional fume hood, or while wearing a fit-tested respirator. MS-222 must be prepared in water similar to the culture conditions of the animal in question. Remember that MS-222 is water soluble and that the culture water must have adequate oxygen, pH, temperature, alkalinity, hardness, and salinity for the animal species being used. MS-222 is a basic compound and must be buffered using sodium bicarbonate. The resulting solution must be between pH 7 and 7.5.

### **LABELING AND STORAGE OF PREPARED SOLUTIONS**

Once prepared, stock containers must be appropriately labeled with the contents and expiration date (within one month of preparation date). If at any time a brown color is observed, properly discard and replace the solution. Solutions must be stored in an opaque container to be adequately protected from light and be subsequently refrigerated or frozen.

### **FEDERAL & ENVIRONMENTAL CONCERNS**

Per the FDA: Do not use within 21 days of harvesting fish for food. Use in fish intended for food shall be restricted to Ictaluridae, Salmonidae, Esocidae, and Percidae. Water temperature should exceed 50 degrees Fahrenheit (10 degrees Celsius). Use of the drug with all other species of fish and cold-blooded animals must be limited to hatchery or laboratory settings.

- If MS-222 is used in the field, all fish must be held for 21 days prior to release.

### **DISPOSING OF MS-222**

Disposal must be consistent with established laws and regulations. Solid waste must be sent off for proper disposal. Solution waste may be diluted at a ratio of 4 parts water to 1 part solution and drain disposed.



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- If in the field, the solution must be diluted 4:1 and disposed of on the ground but not near waterways.

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### Humane Endpoints

### **GUIDELINES REGARDING THE USE OF HUMANE ENDPOINTS**

#### **PURPOSE:**

The purpose of this document is to establish general expectations for the development and use of humane endpoints in studies involving animals in research and teaching at Liberty University.

#### **GUIDELINES:**

##### **PI Expectations**

Per the Guide, the PI should “identify, explain, and include in the animal use protocol a study endpoint that is both humane and scientifically sound” (The Guide, p. 27).

##### **IACUC Expectations**

The IACUC is expected to review each study on a case-by-case basis with regard to the use of humane endpoints. Each review should be study-specific as to species, methodology, and other necessary considerations, as applicable.

Per *the Guide*, the following criteria should be considered for each study, as applicable:

Information that is critical to the IACUC’s assessment of appropriate endpoint consideration in a protocol includes precise definition of the humane endpoint (including assessment criteria), the frequency of animal observation, training of personnel responsible for assessment and recognition of the humane endpoint, and the response required upon reaching the humane endpoint. An understanding of preemptive euthanasia (Toth 2000), behavioral or physiologic definitions of the moribund state (*ibid.*), and the use of study-specific animal assessment records (Morton 2000; Paster et al. 2009) can aid the PI and IACUC when considering or developing proposed endpoints. (The Guide, pp. 27-28).

According to *the Guide*, the IACUC should adhere to the following regarding pilot studies:

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, and veterinarian. A system for communication with the IACUC should be in place both during and after such studies. (The Guide, p. 28).

If a pilot study is warranted, the PI must remain in direct contact with the IACUC Office and veterinarian via email, and if warranted, by phone. Records of any communications should be maintained by the IACUC office.

# LIBERTY UNIVERSITY

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### Injections in Laboratory Animals

## GUIDELINES REGARDING THE USE OF INJECTIONS IN LABORATORY ANIMALS

### PURPOSE:

The purpose of this policy is to provide standardized methods for injecting common laboratory animal species. This policy provides an overview of needle sizes, routes of administration, and maximum allowable fluid volume that can be administered per route.

### GUIDELINES:

The following guidelines outline various circumstances under which injections may be made, and what material is permissible.

### **LIQUID INJECTABLE MATERIAL**

- **Experimental Compounds:** the liquid diluent or vehicle used for injection of cells, infectious agents, or experimental compounds must be sterile to avoid inadvertent introduction of microbial or toxic contaminants into animals, as well as the potential introduction of unwanted variables.
- **Contamination:** if multiple doses are drawn into a syringe for administration to multiple animals, and the syringe is subsequently refilled for dosing additional animals, do not refill the syringe by inserting the used/contaminated needle into the sterile vial. Be sure to use a new, sterile needle when withdrawing solutions from the sterile vial. Do not leave an open needle in the vial as this can lead to contamination of the solution.
- **Veterinary Drugs and Compounding:** anesthetics, analgesics, tranquilizers, injectable saline, and other biologics to be injected into animals must be commercially available pharmaceutical grade compounds. Compounding and use of non-pharmaceutical grade drugs and biologics must be used in consultation with the attending veterinarian per IACUC policy.
- **Preparation of Injectable Agents:** if administering large volumes via subcutaneous or intraperitoneal routes, or if administering intravenous solutions, it is recommended that the fluids are warmed to near body temperature. Refrigerated or room temperature solutions may cause hypothermia in small animals following injection and could lead to the death of the animal.
- **Dilution of Injectable Agents:** some drugs are labeled for intravenous use. In small rodents, IV injections may be difficult due to the small size of the animal's veins. Drugs or compounds intended for intravenous use may be irritating or cause tissue damage if administered by other routes. Therefore, such solutions must be diluted in pharmaceutical grade sterile saline prior to use. Adhere to injectable volume recommendations when making dilutions.

### **INJECTIONS**

Methods for injections include: intradermal (ID), subcutaneous (SC), intramuscular (IM), intraperitoneal (IP), intravenous (IV), intraosseous (IO), or retroorbital. Injection of compounds can pose several risks to the animal, including the potential for disease, injury, or infection. The operator also assumes risk of injury when handling injectable agents, so proper training must be

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given. Risks include injury via struggling or biting animals or accidental self-injection. Do not insert needles to their hubs unless indicated.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### MULTIPLE USES OF A NEEDLE

Sterile hypodermic needles are intended for a single use before being discarded. The LU IACUC has chosen to limit the use of a single needle for injection of up to 5 rodents that are housed in a single cage. In order to minimize the potential for spreading infectious particles among rodents, investigators shall not use the same needle to inject rodents in different cages. Needles used for blood withdrawal or intravenous injection may not be used on more than one animal. Needles must be visually inspected for burrs or other defects after each injection and replaced with a new sterile needle if damage is observed.

**TABLE I. STANDARD VOLUME AND NEEDLE SIZE RECOMMENDATIONS FOR INJECTABLE SITES IN RODENTS AND RABBITS\***

Species	Intradermal (ID) <sup>1,2</sup>		Subcutaneous (SC) <sup>3,5</sup>		Intramuscular (IM) <sup>** 3,5</sup>		Intraperitoneal (IP) <sup>3,5,6</sup>		Intravenous (IV) <sup>2,5</sup>		Intraosseous (IO) <sup>***</sup>	
	Max Volume (ml) per site	Needle Size (gauge)	Volume Range (ml)	Needle Size (gauge)	Max Volume (ml) per site	Needle Size (gauge)	Volume Range (ml)	Needle Size (gauge)	Max Volume (ml)	Needle Size (gauge)	Max Volume (ml)	Needle Size (gauge)
	Needle Length: 1/2-5/8"		Needle Length: 5/8-1"		Needle Length: 1/2- 5/8" GP/Rabbit: 5/8-1"		Needle Length: 1/2-5/8" Rabbit: 3/4-1"		Needle Length: 1/2-5/8" Rabbit: 5/8-1"		Needle Length: 1/2-5/8" Rabbit: 3/4-1"	
<b>Mouse</b>	0.05	27-30	2-3	25-27	0.05	25-27	2-3	23-27****	0.20	26-30	0.20	25-27 <sup>8</sup>
<b>Rat</b>	0.05	25-27	5-10	23-25	0.30	25-27	5-10	23-25	0.50	21-23	0.50	23-26 <sup>9</sup>
<b>USDA Regulated</b>												
<b>Gerbil</b>	0.05	27-30	1-3	25-27	0.05	25-27	2-3	23-25	0.10	27-30	0.10	23-25 <sup>10</sup>
<b>Hamster</b>	0.05	25-27	3-4	25-27	0.10	25-27	3-4	23-25	0.30	25-27	0.30	23-25 <sup>10</sup>
<b>Guinea Pig</b>	0.05	25-27	5-10	23-25	0.30	25-27	10-15	23-25	0.50	25-27	0.50	22-25
<b>Rabbit</b>	0.10	25-27	30-50	21-25	0.50-1.0	23-25	50-100	21-23	1-5	23-25	1-5	20-22 <sup>11</sup>

\*Retroorbital injections in mice: max volume <0.15ml. Needle size: 27 gauge.<sup>4</sup>

\*\*Irritating substances like ketamine must not be administered IM as they may lead to self-mutilation of the affected limb if muscle or nerve damage occurs.

\*\*\*Anesthesia is recommended for intraosseous injections. Injections must be given slowly and maximum volumes are those of IV injections.

\*\*\*\*Recommended range in Formulary for Laboratory Animals, Third Edition is 25-27 gauge.<sup>2</sup> Jackson Laboratories training modules recommend 23-26 gauge.<sup>7</sup>

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## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

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### Recordkeeping and Perioperative Care and Monitoring

## **GUIDELINES REGARDING RECORDKEEPING AND PERIOPERATIVE CARE AND MONITORING**

### **PURPOSE:**

In order to ensure adequate veterinary care, LU requires that appropriate medical records are kept to monitor operative care for research animals.

### **GUIDELINES:**

The Principal Investigator is responsible for ensuring that the appropriate medical records are maintained for the animals involved in research. Any questions regarding the standards of animal care should be directed to the Attending Veterinarian.

### **MONITORING ANESTHETIC USE**

Depending on the species, complexity, and nature of the surgical procedure, the level of monitoring for procedures involving anesthesia can vary. Generally, the higher the potential for pain, distress, complexity, duration, or likelihood of a failed outcome, the higher the need for detailed monitoring.

#### *Monitoring Techniques:*

- Respiration
- Depth of anesthesia
  - Response to stimuli/reflexes
- Skin color
- Mucous membrane
- Blood oxygen saturation
- Heart rate
- ECG
- Blood Pressure

Any monitoring that takes place must be done so until the animal has fully recovered from anesthesia. Reasonable post-operative care including observation of the surgical site, appetite, and overall well-being must be performed. Any procedures involving anesthesia shall be done so with proper veterinary consultation.

### **METHODS OF RECORDKEEPING**

All aspects of the surgery must be recorded and monitored appropriately. This includes pre-operative assessment, anesthetic monitoring, post-anesthetic monitoring, and post-operative care. While a template is available for use on the IACUC website, personnel are encouraged to keep records in a manner that is most appropriate for the species and nature of the work.

Depending on the species, records may be kept individually or for a group of animals. For records on groups of animals, they must all be having the surgical procedure on the same day. Records must be kept in the animal housing room for ease of access.

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### WHAT TO INCLUDE IN RECORDS

#### *Surgical Records*

1. PI/Surgeon Responsible
2. IACUC Protocol Number
3. Species of Animal
4. Date of Procedure
5. Procedure Performed/Used
6. Any anesthetic agent used/route of administration (include dose and duration)
7. Any other drugs used/route of administration (include dose and duration)
8. Any noted complications involving anesthesia, surgery, or drug administration
9. Recovery observations
10. If using a group record, the total number of animals in the group
11. Any deviation from protocol

#### *Post-Op Records*

1. Any drugs used/route of administration
2. Observation of the incision site, activity, pain, excrement, appetite, etc.
3. Record of any suture removal

For additional guidance, refer to the *Guide for the Care and Use of Laboratory Animals* or the Animal Welfare Act and Regulations.



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### Reporting Adverse Events

## **GUIDELINES ON REPORTING ADVERSE EVENTS**

### **PURPOSE:**

To establish and define the system for reporting adverse events regarding IACUC protocols.

### **GUIDELINES:**

The following guidelines serve to outline the process for reporting adverse events on an animal use protocol.

### **WHAT IS AN “ADVERSE EVENT”?**

The Liberty University IACUC has defined an adverse event as any event that is not expected or is not specifically included in the approved IACUC protocol that occurs on a protocol.

### **EXAMPLES OF REPORTABLE ADVERSE EVENTS**

- Unexpected death of a study animal
- Unexpected clinical signs of pain, discomfort, or illness
- Unauthorized access to study animals or facilities
- Escaped animals
- Facility issues (flooding, power outages, temperature extremes, HVAC interruptions)
- Administration of unapproved substances
- Administration of substances beyond the approved range
- Unapproved procedures performed
- Excess samples collected (beyond approved amount or occurrences)
- Unapproved euthanasia performed or failure to confirm death

### **REPORTING PROCESS**

1. Immediately contact the attending veterinarian if an event is ongoing or has the potential to impact the health and welfare of animals or personnel.
2. All adverse events must be reported to the IACUC within 48 hours of the event by emailing [iacuc@liberty.edu](mailto:iacuc@liberty.edu) with a completed Adverse Event Report Form.
  - a. If appropriate for the health and well-being of the laboratory animals, consult a veterinarian regarding their care.
3. The Principle Investigator (PI) is solely responsible for reporting adverse events.
4. Once the IACUC receives the report, it will be reviewed in its entirety, and additional information may be requested.
5. The attending veterinarian along with the IACUC chair will determine any necessary actions to take.
6. The IACUC will relay any required actions to the PI and work to ensure tasks are accomplished in a timely fashion.
7. The PI will be informed in writing once no further action is required.

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### Restraint in Animal Teaching and Research

## **GUIDELINES ON THE USE OF RESTRAINT IN ANIMAL TEACHING AND RESEARCH**

### **PURPOSE:**

To define when restraint is appropriate and inform researchers of the acceptable uses of restraint on animal use protocols.

### **GUIDELINES:**

Any deviation from these guidelines must be scientifically justified and approved by the IACUC.

### **DEFINITION OF RESTRAINT**

Restraint is the use of manual, mechanical, or other means to limit some or all of an animal's normal movement for purposes including sample collection, examination, experimental manipulation, and drug administration. In most research applications, animals are only restrained for brief periods. If possible, animals should be trained through positive reinforcement to present limbs or remain immobile for brief procedures.

### **PROLONGED RESTRAINT**

Prolonged restraint implies a period of extended restraint, during which animals might experience physical or psychological distress or discomfort. The definition of prolonged restraint may vary from species to species, depending on the nature of the research and must be avoided unless scientifically necessary for the research process. Approval by the IACUC will require scientific justification for such practices, a description of the restraint device(s) or method(s), duration of restraint, and monitoring procedures and methods to minimize distress to the animal.

### **RESTRAINT DEVICES**

If it is determined that restraint devices are required, they must be appropriately sized and designed and remain operational such that discomfort or injury to both the animal and research staff is minimized. Restraint devices will be reviewed by the Institutional Animal Care and Use Committee (IACUC) and identified in the protocol with a description of the device and the duration of restraint.

Note: Certain circumstances in which wild animals will be held or captured do not directly qualify as restraint devices for the purposes of this policy. Such devices shall only be used during the period of time necessary to collect or measure the animals. When submitting a protocol, the PI must indicate and specify trap monitoring frequency.

### **IMPORTANT GUIDELINES FOR RESTRAINT OF ANY DURATION**

- Restraint devices must be specifically designed to accomplish research goals that are impossible or impractical by other means or to prevent injury to animals or personnel. They must not be used for convenience.
- Restraint shall only be performed for the minimum time necessary to accomplish objectives.
- If possible, animals should be acclimated to the restraint device and personnel. If animals fail to show progression during this process, the animal must be excluded from the study.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Observation of the animals must be made at appropriate intervals.
- If lesions or illness develop as a result of restraint, veterinary care shall be provided. The observation of lesions, illness, or drastic behavioral change as a result of restraint may necessitate temporary or permanent removal of the animal from restraint.
- The purpose of restraint and its subsequent duration must be explicitly stated when training personnel involved in the study,
- Alternatives to restraint must be considered when developing the study protocol.

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## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### USDA Pain and Distress Categories in IACUC Protocols

## **GUIDELINES REGARDING USDA PAIN AND DISTRESS CATEGORIES IN IACUC PROTOCOLS**

### **PURPOSE:**

Liberty University adheres to the four reportable pain categories defined by the USDA, (B, C, D, and E). These guidelines serve to help researchers in categorizing animal use for IACUC approved protocols.

### **GUIDELINES:**

Any IACUC protocol submitted for approval must classify the potential for pain or discomfort according to these levels, defined as follows (per the Animal Welfare Act 2.36 (b)(5-8):

### **CATEGORY B**

Animals that are bred, held, or acclimated for teaching, use in research, experiments, or surgery that have not yet been used for such purposes.

### **CATEGORY C**

Animals that have been engaged in teaching, research, experimentation, or testing, but experienced no pain, distress, or pain-relieving drugs. Routine procedures (e.g., blood sampling, injections) shall be classified into this category.

### **CATEGORY D**

Animals that have been engaged in teaching, research, experimentation, testing, or surgery that involved pain or distress to the animal for which appropriate analgesic, anesthetizing, or tranquilizing drugs were used.

### **CATEGORY E**

Animals that have been engaged in teaching, research, experimentation, testing, or surgery for which pain or distress was not alleviated by the use of an appropriate analgesic, anesthetizing agent, or tranquilizing drug per potentially adverse effects on procedures, results, interpretations, teaching, research, testing, or surgery.

### **APPROACHES FOR CLASSIFYING PAIN AND DISTRESS**

1. Comparison with Humans
  - a. Consider equivalent or comparable procedures or states in humans and assess whether it would cause more than minimal or transient pain or distress.
  - b. If pain is an expectation, is it necessary to treat, and if so, how?
  - c. What are the potential consequences of not treating the pain?
2. Objective Signs of Pain and Distress in Animals
  - a. Are there any directly observable signs of pain or distress following the procedure?
    - i. Signs may include:
      1. Changes in activity level, appearance, temperament, feeding behavior, physiology
      2. Vocalizations

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INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

3. Surgical site appearance

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## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

**TABLE I. USDA PAIN CATEGORIES AND CORRESPONDING EXAMPLES**

USDA CATEGORY B	USDA CATEGORY C	USDA CATEGORY D	USDA CATEGORY E
Breeding or holding colony protocols	No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanized for tissues; just observed under normal conditions; positive reward projects; routine injections and/or blood sampling.	Pain or distress appropriately relieved with anesthetics, analgesics, and/or tranquilizer drugs or other methods for relieving pain or distress.	Pain or distress or potential pain that is <b>not</b> relieved with anesthetics, analgesics, and/or tranquilizer drugs or other methods for relieving pain or distress.
	<b>EXAMPLES</b>	<b>EXAMPLES</b>	<b>EXAMPLES</b>
	<ol style="list-style-type: none"> <li>1. Holding or weighing animals in teaching or research activities</li> <li>2. Injections, blood collection or catheter implantation via superficial vessels.</li> <li>3. Tattooing animals.</li> <li>4. Ear punching of rodents.</li> <li>5. Routine physical examinations</li> <li>6. Observation of animal behavior.</li> <li>7. Feeding studies which do not result in clinical health problems</li> <li>8. AVMA approved humane euthanasia procedures.</li> <li>9. Live trapping.</li> <li>10. Positive reward projects</li> </ol>	<ol style="list-style-type: none"> <li>1. Diagnostic procedures such as laparoscopy or needle biopsies.</li> <li>2. Non-survival surgical procedures.</li> <li>3. Survival surgical procedures.</li> <li>4. Post-operative pain or distress.</li> <li>5. Ocular blood collection in mice.</li> <li>6. Terminal cardiac blood collection.</li> <li>7. Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with decreased appetite or activity level, adverse reactions, to touch, open skin lesions, abscesses, lameness, conjunctivitis, corneal edema and photophobia.</li> <li>8. Exposure of blood vessels for catheter implantation.</li> <li>9. Exsanguination under anesthesia.</li> <li>10. Induced infections or antibody production with appropriate anesthesia and post-op/post-procedure analgesia when necessary.</li> </ol>	<ol style="list-style-type: none"> <li>1. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs.</li> <li>2. Ocular or skin irritancy testing.</li> <li>3. Food or water deprivation beyond that necessary for ordinary pre-surgical preparation.</li> <li>4. Application of noxious stimuli such as electrical shock if the animal cannot avoid/escape the stimuli and/or it is severe enough to cause injury or more than momentary pain or distress.</li> <li>5. Infliction of burns or trauma.</li> <li>6. Prolonged restraint.</li> <li>7. Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes.</li> <li>8. Use of paralyzing or immobilizing drugs for restraint.</li> <li>9. Exposure to abnormal or extreme environmental conditions.</li> <li>10. Psychotic-like behavior suggesting a painful or distressful status.</li> <li>11. Euthanasia by procedures not approved by AVMA.</li> <li>12. Use of Freund's Complete Adjuvant.</li> </ol>

(Note: There is no USDA pain Category A.)

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### **GUIDELINES FOR DETERMINING USDA CLASSIFICATION IN PROTOCOLS INVOLVING TISSUE COLLECTION BEFORE OR AFTER EUTHANASIA AND/OR ANIMAL PERFUSION:**

- If an animal will be euthanized by an approved physical or chemical method of euthanasia solely for the collection of tissues (after the animal's death), the procedure shall be classified as USDA Pain Category C.
- If an animal will be anesthetized so that non-vital tissues can be collected (liver biopsy or skin biopsy), and the animal will then be allowed to recover, the procedure shall be classified as USDA Pain Category D (survival surgery).
- If an animal will be anesthetized so that non-vital tissues can be collected (liver biopsy or skin biopsy, etc.); and the animal will be euthanized, the procedure shall be classified as USDA Pain Category D (non-survival surgery). In this scenario, it may be necessary to justify why the animal couldn't be euthanized (USDA Pain Category C) rather than anesthetized.
- If an animal will be anesthetized so that vital tissues can be collected (heart, both kidneys or lungs, whole liver, etc.), the animal will obviously succumb to the procedure. To determine whether this will be euthanasia or non-survival surgery, we must consider the definition of euthanasia. A critical component of this definition is "rapid unconsciousness followed by loss of cardiac, respiratory, and brain function." Based on this definition, procedures that require tissue manipulation or other prolonged techniques prior to the animal's death (more than a few minutes) shall be classified as non-survival surgery (USDA Pain Category D). Similarly, if an animal will be anesthetized so that the tissue can be collected in the "freshest" possible state (i.e., heart) and the tissues will be rapidly excised, the procedure shall be classified as euthanasia (USDA Pain Category C). (Note: In this scenario, it is difficult to justify why the animal couldn't be euthanized rather than anesthetized.)
- If an animal will be anesthetized so that it can be chemically perfused, the same "test of time" applies (i.e., long, technical manipulations shall be classified as USDA Pain Category D while rapid intravascular injection of the perfusate without other manipulations shall be classified as USDA Pain Category C).

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Use of Expired Products

### **GUIDELINES REGARDING THE USE OF EXPIRED PRODUCTS**

#### **PURPOSE:**

This policy serves to outline situations in which the use of expired products may be appropriate, and under what circumstances these materials are not to be used.

#### **GUIDELINES:**

USDA Policy states the use of expired materials without justification is considered inadequate veterinary care [9 CFR, 3, 3.110]. Since it is recognized that in some specific situations there may be a rationale to use expired materials, the IACUC has established the following guidelines for the conditional use of expired medical products.

All expired materials must be clearly and individually labeled as “Expired - for acute use only” and kept together in a labeled area physically separate from all other medical materials and drugs.

#### **GUIDELINES FOR SURVIVAL STUDIES**

Expired medical materials may not be used on animals in survival studies. Requested exceptions to this policy must be included in the animal use protocol, along with the scientific justification for the use of expired materials. IACUC approval must be granted prior to use. It is never acceptable to use outdated anesthetics, analgesics, antibiotics or emergency drugs in any case.

#### **NON-SURVIVAL STUDIES**

Expired medical materials such as wraps and sutures may only be used on anesthetized research animals and the animals may not recover from the anesthetic procedure. Anesthesia for these terminal studies must be induced and maintained using current, non-expired drugs.



# LIBERTY UNIVERSITY

INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Veterinary Drug Labeling

## **GUIDELINES ON VETERINARY DRUG LABELING**

### **PURPOSE:**

Research and instructional animals owned by Liberty University may require that drugs be prescribed by a veterinarian for treatment or management of an illness or condition unassociated with research or instructional activities. This document serves as a guide for proper labeling and storage.

### **GUIDELINES:**

Drugs prescribed by a veterinarian for treatment or management of an illness unassociated with research or instructional activities must be labeled and stored appropriately. This includes any drugs prescribed in an extra-label manner.

### **LABEL INFORMATION**

The following information must be included on any prescribed drug label:

- Name and address of veterinarian and facility
- Name of client
- Identification of the treated animal(s)
- Name, active ingredient, quantity of the drug
- Drug strength
- Dosage and duration of treatment
- Administration
- Any necessary cautionary statements
- Slaughter withdrawal or milk withholding times
- Date dispensed

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Policies

#### Animal Escape and Notification

### **POLICY REGARDING ANIMAL ESCAPE AND NOTIFICATION**

#### **PURPOSE:**

Liberty University is charged with specific responsibilities pertaining to the use of animals for teaching and research purposes, including:

- Adequate and humane housing, care, and use of animals as dictated by federal law and policy under the Animal Welfare Act, PHS Policy, and Guide for the Care and Use of Laboratory Animals
- Oversight of animal care and use by a veterinarian with applicable laboratory animal training and experience
- An Institutional Animal Care and Use Committee that oversees the approval of animal research protocols to ensure the integrity of the university regarding animal research
- An environment that is acceptable for those working in animal research, such that physical and biological hazards are minimized or eliminated

The following policy outlines Liberty University's stance on minimizing the likelihood of animal escape and assures that appropriate measures are in place.

#### **POLICY:**

Any animal that escapes its designated holding facility and poses a risk to public safety, health, or personal property, or escapes into a residential or highway area, must be reported to the Liberty University Police Department and the Department Head from which the animal escaped. Further, the attending veterinarian must be notified, who will then communicate with appropriate university officials to engage a community liaison. Failure to implement and uphold this policy will result in review by the IACUC, with the potential for withdrawal or suspension of the study protocol or animal use privileges.

#### **PREVENTATIVE MEASURES**

The Liberty University IACUC is mandated with inspecting animal housing facilities on a semi-annual basis. During this inspection, any deficiencies shall be reported to the Institutional Official and must subsequently be resolved. The IACUC may inspect more problematic areas on a more frequent basis if it is determined necessary for the safety and well-being of the personnel or animals. Any financial responsibility for ensuring adequate animal storage facilities and/or cages rests with the department that operates the facility. The IACUC cannot be denied access to a facility when assessing housing integrity though legitimate requests for postponement may be considered. Each department is responsible for training its personnel in appropriate animal handling and restraint techniques to ensure the likelihood of escape is minimized or eliminated. The IACUC may assist in developing prevention measures and post-escape contingency procedures.

#### **RESPONSE TO AN ESCAPED ANIMAL**

The Department: Each department shall have a designated individual with the responsibility of informing the LUPD and attending veterinarian of any animal escape that meets the criteria established in this policy. This person will also be responsible for assisting LUPD in the

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

recapture and/or return of the animal to its designated holding facility. The department must use any and all means necessary to quickly recapture and return the animal. Any corrective measures that must be performed to secure the animal housing facility must be done so immediately to prevent further escape.

### **LUPD**

Once notified of the escape of an animal covered by this policy, LUPD will determine, after consultation with appropriate personnel, the necessary course of action and any escalation to outside resources.

### **Attending Veterinarian**

The Attending Veterinarian shall make recommendations for correcting the deficiencies that led to the animal's escape. The attending veterinarian must also be available for public comment if deemed necessary by the university public relations officer.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Avian Embryo Use

### **POLICY REGARDING THE USE OF AVIAN EMBRYOS**

#### **PURPOSE:**

The purpose of this policy is to establish procedures for the use of avian embryos in research, teaching, or testing activities at Liberty University.

#### **POLICY:**

Avian embryos are not considered live animals by the applicable US regulatory agencies. However, the scientific community has determined avian embryos beyond half way to hatching can experience pain. If the avian embryos hatch, then they become live vertebrate animals, and as such, are subject to IACUC oversight. To ensure adequate animal care, the IACUC has developed the following policy regarding avian embryos, with chick embryos serving as the model species.

- All personnel who intend to use avian embryos should notify the IACUC using the Avian Embryo Use form. This form allows the IACUC to have a record of the embryo use. Projects using embryos that are euthanized before or equal to 50% development do not require IACUC review. Projects using embryos beyond 50% development must be reviewed and approved by the IACUC prior to initiation.
- Chick embryos younger than embryonic day 10.5 (E10.5) are assumed to be unable to experience pain. Embryos E10.5 or younger should be euthanized by hypothermia, often achieved by placing the eggs in a -20C freezer for at least 4 hours.
- Chick embryos older than embryonic day 10.5 (E10.5) can experience pain and should be euthanized using a rapid and humane method. A suggested method is decapitation.
- Any avian embryos beyond 50% development must be humanely euthanized using CO<sub>2</sub>, anesthetic agents, or decapitation. Remember, embryos are resistant to CO<sub>2</sub> so if exposed to this method, the embryos must be exposed to 90% CO<sub>2</sub> for at least 20 minutes. Dry ice is not an acceptable source of carbon dioxide for this situation.
- Inadvertent hatching may occur. In such cases, investigators are asked to describe their intended method for care or euthanasia of the hatchlings.

**Note:** The common days to hatch for a chicken is 21 days. Other common days to hatch can be found by following this link: <https://extension.psu.edu/incubation-period-of-other-species>

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Breeding of Rodents

## **POLICY REGARDING THE BREEDING OF RODENTS**

### **PURPOSE:**

Maintaining a breeding colony in a research setting may lead to unique scientific and animal welfare concerns. This document serves to outline the process for establishing and maintaining a rodent breeding protocol at Liberty University.

### **POLICY:**

*While maintaining an active breeding protocol, the Principal Investigator must:*

- Coordinate and ensure appropriate space is allocated for the maintenance of a breeding colony.
- Manage rodent colonies consistent with the procedures outlined in an approved IACUC protocol.
- Designate a colony manager (person who has received specific training or has experience managing rodent breeding colonies).
  - This person shall serve as the primary contact regarding any breeding protocol concerns.
- Maintain appropriate colony records. The records must include the following (at minimum):
  - Mating Pairings
  - Generations
  - Number of animals produced
  - Number of animals transferred to protocols (to ensure no excess animals are used)

*While maintaining an active breeding protocol, the designated colony manager must:*

- Separate animals according to approved cage space allocations (to avoid overcrowding)
- Maintain appropriate colony records
- Ensure adequate care is given to all animals

Problems with colony management or breeding must be reported to the IACUC immediately.

### **BREEDING PROCEDURES**

The PI and/or colony manager is responsible for monitoring pregnancies within rodent colonies. Liberty University has established two acceptable breeding methods:

1. Monogamous Pairs (One Male + One Female)
  - a. Only one male per cage for 12 days (allows 2 cycles for impregnation)
  - b. Nesting material must be provided in the cage
  - c. Once mice are separated, female must be monitored daily for weight gain and delivery.
  - d. Litters must be weaned at 4 weeks
    - i. Note: Litters are born approximately 21 days apart
  - e. Post-partum estrus occurs within 24 hours of parturition.
    - i. Males must be removed 12 days after pairing so that they will not be in the cage at this time.
2. Harem Mating (One Male + Two-Three Females)

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- a. Only one male per cage for 12 days (allows 2 cycles for impregnation)
- b. Pregnant females must be removed from their cages and placed in separate birthing cages with appropriate nesting material.
  - i. Only one nursing litter is allowed per cage
  - ii. Pups are nursed for 21 days and up to 28 days (with IACUC approval)
  - iii. Once pups are weaned, the female may be placed back in the harem cage

### **ROUTINE HEALTH CHECKS**

Routine health checks are required as part of an active breeding protocol. Health checks must be performed as follows:

1. On a daily basis, the PI and/or colony manager must check for pregnancies and births
2. Health checks must be documented
3. If new litters are born, cages must be flagged with new litter cards
  - a. Include the date of birth
  - b. Include the projected weaning date

### **AFTER-BIRTH PROCEDURES**

The after-birth process requires that the following procedures be followed:

1. Cages must be left undisturbed for at least three days, except for the following:
  - a. Food and water replenishment
  - b. Overly soiled or wet bedding
    - i. Females must be transferred before the litter
    - ii. A small amount of the original bedding shall be transferred to the fresh cage to allow pups to familiarize themselves with the scent

### **WEANING**

The weaning process requires that the following procedures be followed:

1. Age
  - a. The weaning age for pups is 21 days. In certain circumstances, weaning may be extended to 28 days (modified/mutant strains).
  - b. For cases in which weaning must extend to 28 days:
    - i. Note on the cage card that the litter will be on “Extended Weaning” along with the expected wean date.
  - c. It is not appropriate, under any circumstance, for a three-week-old litter to remain in a cage with a lactating female and her pups.
2. Separation of Sexes
  - a. Male and female pups are separated at weaning. Mice of each sex shall be placed in separate cages.
  - b. If a litter contains only one pup of a given sex, the pup must be housed with others of the same sex. Newly weaned pups must not, under any circumstance, be singly housed.
    - i. A single female pup may remain with the mother.
    - ii. A single male pup may be placed with other male pups from a different litter of the same age.
    - iii. If the parents are a monogamous pair, a single male pup may be housed together with the father in a new cage.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- iv. A single male pup may be housed with female siblings up to six weeks of age (adulthood).
  1. More than one male pup may not be housed with female siblings.
- c. Sexing of the pups must be verified after one week to ensure appropriate separation has occurred.
  - i. This is performed by determining anogenital distance. For females, this distance is 1/3 to 1/2 the distance of the male.
3. Feeding
  - a. At the time of weaning, rodent feed must be placed on the cage floor to span seven subsequent days.

### **OVERCROWDING**

Cages must be monitored daily for pregnancy and birth. Cages that are overcrowded must be dealt with immediately upon notification.

1. If overcrowding is noted, the PI and/or colony manager has 72 hours (including weekends and holidays) to address the issue. If the issue is not addressed within this time frame, the IACUC will be notified and may suspend the protocol.
2. Fighting or wounded rodents must be separated as quickly as possible.
3. When a harem housed female is noticeably pregnant, she shall be separated within 48 hours following the initial observation. If the female is about to give birth, immediately separate her from the harem.
4. If two litters are in the same cage, they must be immediately separated.
  - a. Older pups must be placed into separate cages with food or gel packs on the cage floors.
  - b. Female and newborn pups may be left in the breeding cages.
5. Keep accurate documentation of any updated cage numbers upon birth or separation.

### **IACUC OVERSIGHT AND REVIEW**

The IACUC will carefully review breeding colony protocols to ensure proper colony management is in place. This will include an in depth review of breeding schemes, weaning ages, and methods for identification of individual animals. The IACUC requires that the number of unusable animals be minimized to the greatest extent possible. PIs are encouraged to work with the IACUC to ensure that unusable animals be made available to other researchers whenever possible. If a species or strain is commercially available, the production of animals on a breeding colony must be scientifically justified. Cost and convenience is not considered a valid justification for developing a breeding colony.

### **BREEDING LIMITATIONS AND JUSTIFICATIONS**

Investigators wishing to establish a breeding protocol must submit an application to the IACUC prior to starting work. All requests to add a breeding protocol to an existing protocol require full IACUC review. Each investigator is limited to a total of six active breeding cages at any given time. Exceptions may be granted on a case-by-case basis by the IACUC. Investigators with transgenic strains may request up to six active breeding cages per strain at any given time. Exceptions may be granted on a case-by-case basis by the IACUC. If there is any question as to whether or not breeding is appropriate for the protocol, contact the IACUC for further clarification.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Food or Water Restriction

### **POLICY REGARDING FOOD OR WATER RESTRICTION**

#### **PURPOSE:**

This policy serves to outline the use of food or water restrictions in animal research at Liberty University. All variations to the standard provisions of ad-libitum food and water must be thoroughly described in the protocol with adequate scientific justification according to the below policy.

Standard definitions for the purposes of this policy are as follows:

- **Scheduled Access:** “an animal consumes as much as desired at regular intervals”, (*The Guide, p. 30*)
- **Restriction:** “the total volume of food or fluid consumed is strictly monitored and controlled”, (*The Guide, p. 30*)
- **Deprivation:** the total withholding of food and/or water

#### **POLICY:**

Per *The Guide*, “the objective when these studies are being planned and executed should be to use the least restriction necessary to achieve the scientific objective while maintaining animal well-being. The committee will take the following into consideration when evaluating such protocols, as outlined in *The Guide (p. 31)*:

- Necessary level of regulation
- Potential adverse consequences of regulation
- Methods for assessing the health and well-being of the animals
- Species, strain, stock, gender, and age of the animal(s)
- Housing, time of feeding, nutritive value, fiber content
- Prior experimental manipulation

Investigators must provide finite physical criteria for removing animals from the study, such as weight loss thresholds or clinical signs of dehydration. If food/water restriction is intended as a motivational tool for conditioned-response research, the use of “a highly preferred food or fluid as positive reinforcement”, (*The Guide, p.31*) should be considered.

Further, appropriate daily health monitoring must be in place, to include the following (*The Guide, p. 32*):

- Body weights shall be recorded at least weekly and more often for animals requiring greater restrictions
- Written records shall be maintained, documenting daily food and fluid consumption, hydration status, and any behavioral or clinical changes

#### References:

*Guide for the Care and Use of Laboratory Animals* – 8<sup>th</sup> Edition (National Research Council, 2011)



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Isolation of Sick Animals

### **POLICY REGARDING THE ISOLATION OF SICK ANIMALS**

**PURPOSE:** This policy serves to outline the procedures for detecting and isolating sick animals used for teaching or research at Liberty University.

**POLICY:**

When animals are identified as sick by research staff, the attending veterinarian should be contacted immediately. The attending veterinarian will collect information regarding the health status of the animal from the Principal Investigator, or by an in person visit.

**COLONY HEALTH RISK**

If, in the opinion of the attending veterinarian, the sick animal presents a health risk to other animals in the facility, he or she will instruct the facility or PI to put the animal into isolation. At this time, the PI and the attending veterinarian will create a plan for treatment or euthanasia, depending on the severity of the illness or condition.

**NON-IMMINENT HEALTH RISK**

If, in the opinion of the attending veterinarian, the sick animal does not present a health risk to other animals in the facility, he or she will instruct the PI or facility to leave the sick animal in its home cage. The PI and attending veterinarian will create a plan for treatment or euthanasia, depending on the severity of the illness or condition.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Survival Surgeries

### **POLICY REGARDING SURVIVAL SURGERIES**

#### **PURPOSE:**

This policy defines the types of surgery used in teaching and research protocols, and describes the criteria by which an animal may undergo multiple survival surgeries.

#### **POLICY:**

The classification of a surgery shall be performed on a case-by-case basis by the IACUC, with input from the attending veterinarian with regard to its impact on the well-being of the animal(s) involved. The following definitions serve as a guide to the types of surgery often performed:

- **Survival Surgery:** a surgery from which an animal will regain consciousness following the procedure. Aseptic technique must be used for all survival surgical procedures in all species.
- **Major survival surgery:** a surgical procedure that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions (9 CFR, 1&2), or involves extensive tissue dissection or transection (*The Guide*). Examples include, but are not limited to, laparotomy, thoracotomy, ovariectomy, nephrectomy.
- **Minor survival surgery:** a surgical procedure that does not expose a body cavity and causes little or no physical impairment. Examples include, but are not limited to, suturing, percutaneous biopsy, lymph node biopsy, laparoscopic oocyte collection.
- **Multiple survival surgery:** more than one survival surgery (either major or minor) on a single animal.

#### **MULTIPLE SURVIVAL SURGERY CRITERIA**

Multiple survival surgery is discouraged, but may be permitted under the following circumstances, if justified and subsequently approved by the IACUC:

- An essential component of a single research project or protocol
- Scientifically justified by the PI: explanation of purpose and procedures, total number of surgeries, frequency of the procedure, period of time between procedures, methods used to minimize pain and distress. References must be provided where available.
- Necessary for the clinical health of the animal(s). Consult with the attending veterinarian if such a procedure is warranted.

#### **LIMITATIONS & CONSIDERATIONS**

The following must be considered when performing surgical procedures:

- Individuals performing survival surgery must be knowledgeable about aseptic surgical techniques and have adequate training and skill to conduct the procedure without causing undue post-operative distress to the animal.
- All survival surgical procedures on non-rodent mammalian species must be conducted in surgical facilities designed for that purpose and approved by the IACUC.
  - A dedicated surgical suite is not required for rodents and non-mammalian vertebrates. However, this is the most desirable option and shall be used if available.
- For USDA-covered animals undergoing multiple survival surgeries in separate, unrelated research protocols, USDA/APHIS approval must be secured. Such approvals must be requested by the IO.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- The IACUC will strictly monitor outcomes to determine whether continuation of multiple-survival surgeries is warranted.
- Some procedures classified as minor may induce significant post-procedural pain, and should be similarly justified if performed multiple times.
- Cost savings is not an acceptable justification for performing multiple survival surgeries.
- The number of survival surgeries must be limited to the minimum number necessary to achieve the research objectives, with consideration given to minimizing pain and distress.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Use of Zebrafish

### **POLICY REGARDING THE USE OF ZEBRAFISH**

#### **PURPOSE:**

The purpose of this policy is to establish definitions of what constitutes a live, vertebrate animal when conducting research or teaching activities with zebrafish (*Danio*). Further, this policy is designed to ensure compliance with the Animal Welfare Act, Public Health Service Policy, and established best practices for zebrafish care and management.

#### **POLICY:**

#### **DEFINITIONS:**

The following definitions<sup>1</sup> shall be enforced when referring to the stages of zebrafish growth and development:

- Embryos
  - $\leq 72$  hours post-fertilization; prior to hatching
- Larvae
  - 3-29 days post-fertilization
- Juveniles
  - 30-89 days post-fertilization
- Adults
  - $\geq 90$  days post-fertilization

#### **IACUC protocols are not required under the following circumstances:**

Research or teaching protocols that explicitly and solely utilize zebrafish embryos that will not survive beyond 72 hours. In cases where an embryo unintentionally survives beyond 72 hours, the investigator must adhere to the below guidance on “resolution of unanticipated hatching.”

The IACUC must approve the use of zebrafish embryos if those animals are expected to survive beyond 72 hours. Zebrafish embryos that are manipulated before the 72-hour mark and which are appropriately euthanized before that time are not regulated by the IACUC. All euthanasia methods must be performed in accordance with current AVMA Guidelines.

#### **IACUC protocols are required under the following circumstances:**

The Liberty University IACUC must evaluate and approve all research or teaching activities in which zebrafish are allowed to develop beyond 72 hours.

- Zebrafish larvae (3-29 days post-fertilization)
- Zebrafish juveniles (30-89 days post-fertilization)
- Zebrafish adults ( $\geq 90$  days post-fertilization)

#### **RESOLUTION OF UNANTICIPATED HATCHING/DEVELOPMENT**

On protocols where investigators are using zebrafish embryos, the possibility exists whereby eggs may unintentionally hatch or survive past 72 hours. In these instances, the IACUC has established the following procedures to ensure compliance with the regulations:

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### **OPTION A: Immediate Transfer to Approved Protocol**

- Should zebrafish hatch or survive past 72 hours on a protocol where such work is not approved, the larvae must be immediately transferred to an IACUC-approved protocol that covers zebrafish use beyond the embryonic stage. If no such protocol exists, the zebrafish must be euthanized (Option B).

### **OPTION B: Euthanasia**

- Should zebrafish hatch or survive past 72 hours on a protocol where such work is not approved, and option A was not possible, the zebrafish larvae must be immediately euthanized using an AVMA approved method. These methods may include<sup>2</sup> immersion in solutions of buffered tricaine methanesulfonate (MS-222), buffered benzocaine, quinaldine sulfate, and 2-phenoxyethanol OR rapid chilling as long as transfer from acclimatized temperatures to water associated with 2 degree to 4 degree ice slurry occurs rapidly with as little transfer of warmer water as possible.

### **OCCUPATIONAL HEALTH CONSIDERATIONS**

Working with Zebrafish may result in exposure to certain risks.

### **REFERENCES**

1. Kimmel et al., 1995. Developmental Dynamics 203:253-310.
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

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INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

## Animal Disposition

### Policies

#### Euthanasia of Laboratory Mice and Rats

## **POLICY REGARDING EUTHANASIA OF LABORATORY MICE AND RATS**

### **PURPOSE:**

Liberty University has a commitment to maintain regulatory compliance as a part of the Institutional Animal Care and Use Program. As such, Liberty University seeks to provide appropriate, humane euthanasia for all laboratory rodents when and where applicable.

### **POLICY:**

It is the policy of Liberty University to adhere to the following guidelines as the minimal acceptable standard for animal care regarding euthanasia. Any form of euthanasia that does not follow the AVMA Guidelines on Euthanasia must be requested and justified in the study protocol during the IACUC approval process. According to “The Guide for the Care and Use of Laboratory Animals”:

*“Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the current [AVMA Guidelines](#) on Euthanasia. In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; irreversibility; time required to induce unconsciousness; appropriateness for the species and age of the animal; compatibility with research objectives; and the safety of and emotional effect on personnel.*

*Euthanasia may be planned and necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments. Criteria for euthanasia include protocol-specific endpoints (such as degree of a physical or behavioral deficit or tumor size) that will enable a prompt decision by the veterinarian and the investigator to ensure that the endpoint is humane and, whenever possible, the scientific objective of the protocol is achieved.*

*The selection of specific agents and methods for euthanasia will depend on the species involved, the animal’s age, and the objectives of the protocol. Generally, chemical agents (e.g., barbiturates, nonexplosive inhalant anesthetics) are preferable to physical methods (e.g., cervical dislocation, decapitation, use of a penetrating captive bolt); however, scientific considerations may preclude the use of chemical agents for some protocols. Furthermore, because neonatal rodents are resistant to the hypoxia-inducing effects of CO<sub>2</sub> and require longer exposure times to the agent (Artwohl et al. 2006), alternative methods should be considered (e.g., injection with chemical agents, cervical dislocation, or decapitation; Klaunberg et al. 2004; Pritchett-Corning 2009).” All methods of euthanasia should be reviewed and approved by the IACUC.”*

### **AS IT RELATES TO EUTHANASIA, LIBERTY UNIVERSITY UPHOLDS THAT:**

- Chambers are not to be pre-loaded with CO<sub>2</sub> when using them for euthanasia. When using a CO<sub>2</sub> chamber for euthanasia, the user must be properly trained and be familiar with the SOP for the process and equipment. All posted instructions must be followed.
- When using an inhalant for the purpose of euthanasia, a secondary method must be used to confirm animal death. Such methods include: cervical dislocation, exsanguination, thoracotomy, or decapitation. The appropriate method for the species shall be used, and must be included in the approved IACUC protocol.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Mechanical euthanasia without anesthesia must be scientifically justified and noted in the IACUC protocol.
- Prior to performing any euthanasia, it is the responsibility of the PI to ensure that all persons are adequately trained in such procedures.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Protocol Closure or Transfer in the Event of a PI's Absence

## **POLICY REGARDING PROTOCOL CLOSURE OR TRANSFER IN THE EVENT OF A PRINCIPAL INVESTIGATOR'S ABSENCE**

### **PURPOSE:**

This document serves to outline the process for handling active protocols in cases where the Principal Investigator has departed Liberty University without closing or transferring the protocol to another approved PI.

### **POLICY:**

In the event that the Liberty University IACUC is notified of the departure or absence of an animal-using PI with active protocol(s), the protocols will be reviewed to determine the status of any animals that remain. This policy extends to all animal users at the University.

### **IF IT IS DETERMINED THAT ACTIVE ANIMALS DO NOT EXIST ON THE PROTOCOL(S), THE FOLLOWING SHALL OCCUR:**

1. The PI must be contacted via email and will be asked to close or transfer the protocol within 10 days.
2. If a response is not received within 10 business days, an email will be sent to the Department Head and other associated personnel on the protocol requesting that appropriate action be taken to either close or transfer the protocol within 10 days.
  - a. If a response is not received within 10 business days, the protocol(s) will be immediately closed by the IACUC, citing "PI has left the University". All personnel on the protocol will be notified of the closure, and all animal research must immediately stop. Any closures as a result of this process will be reported to the IACUC during the next scheduled meeting.

### **IF IT IS DETERMINED THAT ACTIVE ANIMALS DO EXIST ON THE PROTOCOL(S), THE FOLLOWING SHALL OCCUR:**

1. The PI must be contacted via email and will be asked to appropriately dispose of the animals and related protocol within 5 days.
  - a. The attending veterinarian must be notified of an existing protocol with no PI oversight and will assume care of the animals in a holding protocol until the IACUC resolves the protocol in question.
  - b. Disposition of the animals may include transferring the animals to a different IACUC approved protocol, or euthanasia as defined in the existing approved protocol.
2. If a response is not received within 5 business days, an email will be sent to the Department Head and other associated personnel on the protocol requesting that appropriate action be taken to either close or transfer the protocol.
  - a. If a response is not received within 5 business days, the issue will be referred directly to the IACUC for guidance and resolution.

If a protocol is to be transferred to another PI, a Change in PI form and New Application for the Use of Animals in Research Form must be completed and submitted to the IACUC.



# LIBERTY UNIVERSITY

INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

## Animal Facilities & Housing

### Guidelines

#### Cage Card System

### **GUIDELINES REGARDING THE VIVARIUM CAGE CARD SYSTEM**

#### **PURPOSE:**

To facilitate animal care and management at Liberty University, the IACUC has developed guidelines on the use of colored cage cards. The below described cage card color system will enable personnel to rapidly identify cage conditions and allows appropriate action to be taken.

#### **GUIDELINES:**

The standard white cage card should be used in most cases (example below). If a colored card is required, these cards should be visibly placed behind the standard white cage card. The only exception to this would be the green breeder card. Green breeder cards should take the place of the standard white cage card when breeder pairs are used.

This system is not meant to replace a PI's individual records, and is designed more toward smaller (rodent) animals. Any questions about the cage card system should be directed toward the vivarium manager or the IACUC Office.

<input type="checkbox"/> PI: _____ (____) _____		Animals in cage: _____
<u><b>Mice</b></u> <input type="checkbox"/> CD-1 <input type="checkbox"/> BALB/c <input type="checkbox"/> A/J <input type="checkbox"/> C57/B6 <input type="checkbox"/> Other _____	<u><b>Rats</b></u> <input type="checkbox"/> Other _____	<u><b>Rabbits</b></u> <input type="checkbox"/> New Zealand White <input type="checkbox"/> Other _____
<b>Protocol #:</b> _____	<b>Source:</b> <input type="checkbox"/> Charles River <input type="checkbox"/> JAX	
<b>DOR:</b> ____/____/____	<b>DOB:</b> _____	<b>Male / Female</b>
<b>Experiment</b> _____	<b>Sacrifice Date:</b> _____	
<b>Veterinarian Contact Info</b> _____		

### **WHITE—STANDARD CAGE CARD**

- The standard white cage card should be visibly displayed on each cage, unless the cage is a breeder cage. Each standard white cage card must contain, at minimum, the following information:
  - PI Name
  - Species
  - Vendor
  - Date of Receipt (DOR)
  - Number of animals

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### **GREEN—BREEDER**

- In breeding colonies, the green breeder cage card takes the place of the standard white cage card. This card should include the breeder IDs and pairing date in addition to the standard cage card information. This enables observers to identify any breeder cages in a prompt manner. For instructions on how to house, care for, and maintain breeding colonies, refer to the IACUC's breeding policy. Breeding colonies must be explicitly approved by the IACUC for reasons other than cost or convenience.

### **BLUE—PUPS/NEW LITTER**

- This card is to be placed behind the green breeder card when a new litter is born. This blue card should track the date of birth and weaning dates for the pups, as well as the number of pups born. Weaning dates are typically 21 days or 28 days (with IACUC approval). More information on weaning and animal counting can be found in the IACUC policies and guidelines handbook.

### **YELLOW—ANIMAL HEALTH ISSUE & VETERINARY RESPONSE**

- This card is to be used if any health issues are identified. An observer should record the issue on the card and promptly inform the PI or vet of the issue. The PI or Vet may then take appropriate action and should record any follow-up actions taken on the back of the yellow cage card.
- **BLACK STICKER—DEAD ANIMAL:** A black sticker is to be placed on a yellow cage card should any animal deaths be observed. The observer should fill out the back of the yellow card with as much information as possible, including time/date of observation, and number of dead animals. Once completed, this yellow card should be placed behind the standard white cage card.

### **“H” DESIGNATION—HOLDING PROTOCOL**

- Any animals that are off-limits to researchers in a holding protocol shall have an “H” visibly written on the standard white cage card. This alerts all individuals that the animals are not to be engaged in research or teaching until they have been transferred from the holding protocol by the Attending Veterinarian.

### **LAVENDER—SPECIAL DIET OR SPECIAL HUSBANDRY**

- This card allows for a description of any special husbandry needs (modified diet, modified food/water schedule). This information will allow for inspectors and staff to understand any observed differences in husbandry or animal behavior.

### **RED—SURVIVAL SURGERY/POST-PROCEDURE MONITORING**

- This card should be used to flag any animal cages in which animals are recovering from surgical procedures. This notifies any observers that the animals are recovering while also detailing any special care that must be provided. The time and duration of the recovery period should be noted on this card (begin, duration, end).

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Tracking Animal Use

### GUIDELINES REGARDING TRACKING ANIMAL USE

#### **PURPOSE:**

The purpose of this guideline is to provide researchers and staff with an understanding of how animals should be tracked at Liberty University.

#### **GUIDELINE:**

All animals generated or obtained for the purposes of research or teaching must be counted against an approved protocol once weaned, ordered, or caught in the wild. The IACUC and federal agencies have the authority to request and review a protocol's animal tracking records at any time. The IACUC strives to maintain a central list of animal use, placing updates from the PIs into a management spreadsheet.

#### **ORDERING/PURCHASING FROM A COMMERCIAL VENDOR**

- All animal orders must be done so via the animal ordering form on the IACUC website. Both the IACUC and the person responsible for ordering animals in your department will be notified of the request.
- Once an order is placed, the number of animals will be deducted from the total number approved on the protocol in the IACUC tracking spreadsheet.

#### **OBTAINING ANIMALS FROM A NON-COMMERCIAL SOURCE**

- Contact the IACUC for approval prior to obtaining such animals. Upon IACUC approval, the total number of animals will be deducted from the number on the approved protocol.

#### **TRANSFERRING ANIMALS BETWEEN PROTOCOLS OR FACILITIES**

- Generally, the IACUC does not allow multiple uses of animals on more than one IACUC protocol. That said, it may be appropriate in certain cases to transfer an animal for use on a second protocol if it is in compliance with IACUC policies and guidelines. USDA approval is required for USDA-covered animal transfers to use an animal under two projects with different scopes.
- An animal transfer request form should be submitted in such cases.
- When an animal transfer request form is received by the IACUC office, the number of animals to be transferred to the receiving protocol is deducted from the protocol. Animals will have already been counted against the donor protocol at the time of ordering, breeding, import, or transfer.
- Should this process be cancelled for any reason, the animal numbers will not change.

#### **BREEDING ANIMALS**

- The PI must have an IACUC approved breeding protocol in place prior to engaging in the breeding of research animals. This protocol must include a description of the breeding methods and an assurance that appropriate documentation and tracking methods are in place for maintaining a colony.
- All rodents must be counted when weaned, unless the animals are being euthanized and utilized for research or teaching activities prior to weaning. Animals that are not the

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

correct genotype/sex/etc. must still be counted against the total number of approved animals on the protocol. Pups that are stillborn do not need to be counted.

- Animals bred but not used for genotyping or experiments must be euthanized prior to weaning or be transferred to another protocol. This should be explicitly stated in any animal use protocol.

### **ANIMALS USED IN FIELD RESEARCH**

- Unless otherwise stated in the approved protocol, animals should be counted at the point of capture whether they are specific to the animal needed for that field assessment or not.
- The PI or designee must keep track of the number of animals used during the field season and should report these numbers on the annual review form. If it is expected that the number approved on the protocol will be surpassed, an amendment must be submitted to the IACUC for approval prior to proceeding.
- Animals captured in the field should not be housed unless specifically approved in the animal use protocol.

### **TRACKING USDA-COVERED SPECIES**

USDA-covered species exclude: fish, birds, reptiles, amphibians, and rats (*Rattus*) and mice (*Mus*) bred for research. USDA-covered species must be tracked by the highest pain category of procedures conducted on them for research. The annual report to the USDA includes the number of animals used under each pain category and should be reported to the IACUC office prior to December.

- All animals should be tracked for inventory purposes (acquisitions, births, deaths, euthanasia, transfers, sales), but the only animals that are required to be reported on the annual report are those used or held for use in research activities.
- Stillborn animals, unless this is study related, are not reportable to the USDA on the annual report.
- If breeding or manipulation for research purposes of pregnant females or their fetuses or offspring is being done on covered animals, then stillbirths or spontaneous deaths would need to be reported.
- Incidents of USDA-covered species that die in transport are reported immediately to the USDA through the VMO, not on the USDA animal report, so that transportation can be evaluated; these animals would not be counted on the protocol.

### **MULTIPLE USES OF ANIMALS**

- The IACUC must review and approve all procedures including multiple procedures on a single animal.
- Multiple survival surgeries must be scientifically justified and approved by the IACUC before implementation. Animals with previous survival surgery on one protocol cannot be used for survival surgery on a second protocol unless approved by the IACUC. USDA approval is required before USDA-covered animals can be transferred for use on two different projects.
- Animals must be counted once against each protocol in which they are used.
- If a terminal procedure is conducted under one protocol, and tissue is taken for use on another protocol, the animal is only counted against the first protocol.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Policies

#### Policy Regarding Animal Husbandry, Care, and Documentation

##### **PURPOSE:**

This policy serves to provide guidance on daily and periodic animal husbandry practices.

##### **POLICY:**

According to the Guide, standard animal husbandry activities must be performed at regular and appropriate intervals, as applicable.

##### **DAILY ACTIVITIES**

- Animal Health & Welfare Walkthrough
- Food and Water Check
- Room Temperature Monitoring
  - Monitoring shall include temperature of time of observation, daily high, and daily low.
- Room Ventilation Check
  - Ventilation check should include air movement, odor, and if applicable, pressure differential or humidity.

##### **PERIODIC ACTIVITIES**

- Cage Space & Overcrowding Check
- Bedding Changes
- Caging Changes
- Room Cleaning & Sanitation
  - Cleaning and sanitation shall include floors, walls, ceilings, fixtures, etc.

The activities listed above are not all encompassing, and should be modified based on environmental and housing conditions, species, health status, etc. Aquatic animals may require additional assessments, care, and/or husbandry tasks.

Current, accurate, and easily accessible documentation of animal care activities is required. Such information is vital to persons involved in the animal care and use program at Liberty University. As such, the Liberty University IACUC requires care and husbandry activities to be maintained on an animal housing room activity log at the animal room level, though this may be modified as appropriate depending on the species involved. An example log is attached to this policy and is available on the IACUC website; this log may be modified as needed. Completed logs must be maintained and provided upon request. Husbandry logs should be maintained for a minimum of three years.

Facility or vivarium managers should review the husbandry logs on a routine basis to ensure accuracy and compliance with husbandry and animal care requirements, policies, and expectations. Facility and site inspections will request these documents.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Failure to maintain and furnish a complete, accurate log results in not compliance with regard to federal and institutional policies. In such instances, the IACUC or other agencies may take additional or required actions to address immediate, emerging, or trending issues.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Frequency:		DAILY														
Date	Time	Health ✓	Water ✓	Feed ✓	Current Temp (°F)	High Temp (°F)	Low Temp (°F)	Vent. ✓	Pressure Diff. (+/-)	Cage Space/ Overcrowding ✓	Bedding Change	Bottle Change	Cage Change	Floor Sweep/Mop	Room Clean/San.	Comments
1																
2																
3																
4																
5																
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**Note:** Individuals completing this log must clearly identify themselves in the comments section. A separate log must be maintained in each animal room.

Animal Identification

**POLICY REGARDING ANIMAL IDENTIFICATION**

**PURPOSE:**

To define a system of identification for animals used in research, teaching, or training, compliant with the Animal Welfare Act and PHS Policy.

**POLICY:**

Liberty University IACUC has established the following policy regarding appropriate animal identification practices.

**FOR RODENTS AND NON-MAMMALIAN SPECIES:**

1. The following information is required for animal identification (at minimum):
  - a. IACUC Protocol #
  - b. PI Name
  - c. Contact Person (If other than PI)
  - d. Species
  - e. Source (vendor, colony, etc.)
2. The animal cage must prominently display the above information. Each animal cage must have its own cage card that uniquely identifies it with respect to any other cages in the study area.
  - a. The animal identification information listed above may be displayed on either:
    - i. Individual cage cards
    - ii. On a sign that clearly pertains to all animals in cages grouped together (on a rack or in the same room, but only if the information is common to all animals)
  - b. Intermixing of cages of animals with different identifying information requires that information be displayed on individual cage cards.
3. If instances where a strain, stock, or mutation is expected to produce a compromised health phenotype (including death), the phenotype or genetic information must be explicitly specified.
4. If animals will require post-procedural health monitoring (post-surgery, special diet or drug administration, viral or radioactive containment), this information, including dates, must be indicated on cage cards through the period of time necessitated.
5. Ear punch, ear tag, microchips, and tattoos are acceptable methods of marking individual animals, and do not require protocol approval.
  - a. Toe-clipping is only allowed with IACUC approval (must have scientific justification) and never after 7 days of age in mice
6. Animals removed from the vivarium (or other means of housing) must be labeled with the time and date of removal, as well as the responsible person.
7. Cages that contain pre-weanling animals must be marked with the date of birth and count of the litters.

**FOR NON-RODENT MAMMALS:**

Except as follows, the above policy also applies to non-rodent mammals.



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

1. The unit of identification is the individual animal. One identifying card per animal must be located at the site where the animal is housed.
2. Each animal must be identified according to applicable federal requirements specific to the species (tattoo number, transponder, medallion, etc.).

### **NUMBERING SYSTEM**

Permanent, unique identification of animals at Liberty University is critical for both regulatory and scientific reasons. Maintaining an accurate numbering system will allow for better conduct, as well as appropriate and humane animal care.

Liberty University IACUC suggests the following numbering system, where every animal (or group of animals) receives a unique number. These numbers:

1. Are easily obtained
2. Increase efficiency and accuracy of census and tracking of animal populations
3. Will help prevent mistakes such as inaccurate transfer of animals, or procedures performed on the wrong animals
4. Meet regulatory requirements for animal identification
5. Provide a mechanism whereby investigators can attach important information to an animal or group of animals

### **ANIMAL NUMBERING SHALL BE PERFORMED IN THE FOLLOWING MANNER:**

PI-Protocol#.Animal#/Cage#→SMITH-1.12

\*Laboratory animals housed in a location other than a university-owned vivarium must be approved by the IACUC.

Larger animals must each have their own sticker. Rodents, fish, and other small animals housed in groups can use one sticker for each animal, or can alternatively use one sticker for each cage or housing unit.

Once established, an identification sticker must not be replaced. After labeling an animal or group of animals, the identification sticker shall remain until the animals or cage are decommissioned from the protocol. Change in information, such as PI or protocol number may require a new identification sticker with an updated protocol number in the animal ID.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Approval of New Animal Facilities

## **POLICY REGARDING THE APPROVAL OF NEW ANIMAL FACILITIES**

### **PURPOSE:**

To detail the process for the completion and inspection of new animal facilities, prior to use for animal housing.

### **POLICY:**

Animal facilities designed to house vertebrate animal species may not begin to house animals prior to completing the required steps for review and approval.

### **BEFORE HOUSING ANY ANIMALS, THE PERSON IN CHARGE OF THE NEW ANIMAL FACILITY MUST:**

1. Review and obtain approval from the attending veterinarian regarding all SOPs for the facility.
2. Review and obtain approval from the attending veterinarian for the personnel staffing plan at the new facility.
3. Review and obtain approval from the attending veterinarian for the animal acquisition plan.
4. Complete an inspection of the facilities by the attending veterinarian.
5. Write all necessary SOPs prior to fully staffing the facility to ensure adequate training and enforcement of policy.
6. Define the status of the facility as either conventional or barrier, and provide the necessary SOPs.
7. Remedy any deficiencies found in the initial inspections prior to staffing the new facility.
8. Hire and train appropriate staff to ensure adequate and responsible care for new animal acquisitions.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Group Housing of Social Species

## **POLICY REGARDING GROUP HOUSING OF SOCIAL SPECIES**

### **PURPOSE:**

Liberty University, in its commitment to comply with all applicable federal and state laws regarding animal care, has developed the following policy regarding the housing of social species.

### **POLICY:**

Unless otherwise justified, social housing shall be the default method of housing at Liberty University. Circumstances in which social housing may be suspended include: social incompatibility, inappropriate behavior, approved husbandry practices, justified veterinary concerns, or scientific necessity that has been previously approved by the IACUC.

Wherever possible, animals shall be housed in pairs or groups of individuals deemed to be compatible. If a circumstance arises in which an animal is not placed into a pair or group, it must only be for the minimum period of time necessary for the activity unless scientifically justified. In the absence of other animals (single housing), enrichment shall be offered to the animal, such as human interaction, periodic exposure to larger enclosures, supplementation, or the addition of a companion animal.

In certain cases, exceptions to the group housing policy may be warranted. Below are common types of exceptions:

### **ANIMAL HUSBANDRY AND MANAGEMENT**

Liberty University IACUC permits single housing of social species for standard husbandry practices, or in cases where animal well-being may be jeopardized. If animals are housed singly on an infrequent basis for justifiable reasons, IACUC approval is not required. However, for cases where single housing will occur on a frequent basis, approval of the IACUC will be required based on scientific justification from the submitted protocol.

### **CLINICAL REASONS**

Due to certain medical concerns, it may be appropriate at times to separate animals for appropriate veterinary treatment. For instances such as this, the IACUC does not need to give approval. The veterinarian who is in care of the animal will record the time spent in single housing in the animal's medical record, and will re-house the animal when it is deemed appropriate to do so. Cases such as this will be reported to the IACUC per the discretion of the attending veterinarian.

### **EXPERIMENTAL CONDITIONS**

Certain experimental factors may require the single housing of certain animal species. In cases such as this, IACUC approval will be necessary, along with scientific justification. The single housing cannot take place until approval is given by the IACUC for the specific protocol.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Holding of Animals not on Active Research Protocols

### **POLICY REGARDING THE HOLDING OF ANIMALS NOT ON ACTIVE RESEARCH PROTOCOLS**

#### **PURPOSE:**

This document serves to outline the process for holding and maintaining research animals not currently assigned to a protocol on a global animal holding protocol.

#### **POLICY:**

Use of the animal holding protocol is intended to be temporary, for a period of no longer than 60 days. During this time, researchers must take the necessary actions to gain approval for their animal use protocol in order to avoid forfeiture of the animals. Situations in which the use of the animal holding protocol may be warranted include:

1. Animals are ordered without an approved protocol in place.
2. Animals originating from inactive, suspended, or terminated protocols (including protocols where a PI is absent or missing).
3. Animals on a protocol under investigation by the IACUC for potential issues of noncompliance where the welfare of the animals is in question.
4. New LU investigators without an IACUC-approved protocol having animals that may require immediate housing at Liberty University.
5. Investigators that are leaving LU and do not have approval for transferring the animals to the new institution.

To request that animals be placed on the animal holding protocol, researchers must complete the Holding Protocol Request Form and submit it to [iacuc@liberty.edu](mailto:iacuc@liberty.edu) for approval.

#### **RESTRICTIONS & CONSIDERATIONS**

- Any fees or costs associated with the use of the animal holding protocol will be charged to the PI or the responsible department.
  - No animal related costs may be charged to federal funding sources in the absence of an approved IACUC protocol.
- Animal cages must be clearly identified as being on the holding protocol (indicated by the inclusion of an “H” at the end of the existing number).
- Animals on the animal holding protocol are strictly off limits to any investigators.

#### **RESEARCHER RESPONSIBILITIES**

The PI must work with the IACUC to ensure that any animal use protocols are active and current for any anticipated or ongoing animal use. It is the responsibility of the researcher to ensure that any necessary approvals are obtained prior to protocol expiration.

#### **IACUC RESPONSIBILITIES**

The IACUC provides a mechanism for animals to be rapidly transferred to a universal holding protocol in cases where it is deemed necessary in order to protect the health and welfare of the animals, or to prevent the waste of research animals.

## **ATTENDING VETERINARIAN**

The AV maintains the global holding protocol in such a manner as to receive and maintain animals that would otherwise be euthanized for lack of an existing active, IACUC-approved protocol.

## **TRANSFER FROM HOLDING PROTOCOL**

Animals may only be transferred from the animal holding protocol to a new or existing protocol upon approval from the attending veterinarian and the IACUC.

## **ANIMAL CARE & MANAGEMENT**

- No experimental procedures may be performed on animals while they are on the animal holding protocol. Any such use of animals on the holding protocol will be treated as a serious regulatory non-compliance.
- Breeding may be performed to maintain viability of specific lines occurring under this protocol. Expansion colony breeding is not permitted.
- Feeding, sanitation, and enrichment will be performed as appropriate for the species.
- No tissues may be utilized from euthanized animals without prior IACUC approval.
- Animals may not be euthanized for research purposes unless they are transferred to an approved IACUC protocol.
- The attending veterinarian must be made aware of any pre-existing conditions prior to receiving animals on the animal holding protocol. This may include:
  - Surgical implants
  - Zoonotic diseases
  - Dietary restrictions or needs
  - Past surgical history
  - Poor fecundity

## **HOLDING DUE TO PROTOCOL EXPIRATION**

If animals are placed onto the animal holding protocol due to the expiration of a protocol, they may remain for no more than one month from the date of expiration. If a renewal protocol has been submitted within the one month period, an additional month may be granted until approval is secured for a total of two months past the original expiration date.

## **HOLDING PROTOCOL EXPIRATION**

In the event animals on the holding protocol have been held beyond the two-month maximum, animals may be euthanized or transferred by the attending veterinarian to an approved protocol.

For studies in which wild animals have been captured and held on the animal holding protocol, it may be appropriate to release the animals in a manner consistent with federal and local laws and guidelines.

Extensions beyond the two-month maximum may be granted on a case-by-case basis by the attending veterinarian.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Housing and Environmental Enrichment for Laboratory Animals

### **POLICY REGARDING HOUSING AND ENVIRONMENTAL ENRICHMENT FOR LABORATORY ANIMALS**

#### **PURPOSE:**

This document serves to assist researchers in developing appropriate enrichment programs for the species used in research, teaching, or training at Liberty University.

#### **POLICY:**

Enrichment programs must be reviewed by the IACUC, researchers, and the attending veterinarian on a regular basis to ensure that they are beneficial to the well-being of the animals, and that the programs are consistent with proper animal use. Deficiencies must be updated to reflect current knowledge and practices. Personnel responsible for the care of animals shall be properly trained in the behavioral aspects of animal care to appropriately monitor the effects of any enrichment, as well as identify the development of any adverse behavior.

#### **DEFINITIONS**

- Standard Housing
  - Housing conditions as provided to animals by default per standard procedures.
- Group Housing
  - More than a single animal within a cage or primary enclosure.
- Social Contact
  - Visual, auditory, olfactory, or tactile contact with others of the same species.
- Isolation
  - Housed individually in a room without visual, auditory, olfactory, or tactile contact with others of the same species.

#### **STANDARDS**

- Each animal housing room maintains a single species at any given time unless special arrangements have been made for compatible species.
- Enrichment and appropriate social contact must be provided according to the table below.
- Changes from the standards listed below are not allowed except under the following circumstances:
  - Changes are described in the applicable protocol approved by the LU IACUC
  - The attending veterinarian prescribes the change
- Provision of enrichment must be provided so as not to cause undue stress or otherwise harm the animal or alter the species appropriate standards for husbandry, nutritional requirements, or housing.
- Physical materials used for enrichment must be easily sanitized to the level required by the building's SOPs.
- Husbandry must be provided to any singly housed mouse, rat, hamster, guinea pig, or other rodent unless otherwise specified (must be approved by the IACUC).
- Materials and labor costs associated with optimal enrichment will be charged to the Principal Investigator.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Enrichment must be conducted in such a way to not interfere with the research results, or must be controlled appropriately.

**TABLE I. ENRICHMENT STANDARDS**

<b>Species</b>	<b>Standard Housing and Enrichment for Group Housed Animals</b>  <i>Requirements for group housed animals. Changes from these standards are not allowed except by IACUC approval.</i>	<b>Enrichment for Singly Housed Animals</b>  <i>Requirements for singly housed animals. Changes from these standards are not allowed except by IACUC approval.</i>	<b>Optional Enrichment</b>  <i>These options are not required to be addressed in the IACUC Protocol. PI must decide which animals receive and arrange for the provision of this enrichment.</i>
Mice Rats Hamsters Guinea Pigs Other Rodents	Group housed whenever possible. Approved polystyrene, polycarbonate, or other high temp solid bottom plastic cage fitted with a functioning filter-top or polyethylene disposable cages. Cages may be connected to racks providing filtered air. Commercially available paper, wood chip, or corn cob type bedding. Wire bar lide, frame, or box feeder. Resting lofts where applicable (i.e. Techniplast caging). Water bottle or lixit for automatic watering. Approved feed in a feed hopper or wire bar lid.	Singly housed animals will be provided with one of the following: <ul style="list-style-type: none"> <li>• Cotton nesting material</li> <li>• Sterilized paper towel</li> <li>• Paper towel (not sterile)</li> <li>• Paper or plastic tubes</li> <li>• Bedding enriched w/ paper nesting chips</li> </ul>	Provision of cotton nesting, sterilized paper, paper or plastic enclosures.
Rabbits	Individually housed, but with social contact with conspecifics whenever possible. Easily sanitized wire bottom caging of the appropriate size for the species. Water bottle or	Single <u>isolated</u> animals will have positive human interaction daily.	Small portions of fruit, vegetables, alfalfa not to interfere with primary diet.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

	<p>lixit for automatic watering at all times. Nutritionally complete pelleted food per weight daily. Enrichment materials that are easily sanitized, (hard balls, plastic chains, PVC pieces, nylabones) will be placed in cages. Small portions of timothy hay or other grass hay, in portions that will not offset normal diet.</p>		
Reptiles/Other Amphibian	<p>Group housed whenever possible in easily sanitized and secured enclosures with a safe heating device located in such a way to allow animal(s) to thermoregulate appropriately for the species. Substrate that is easily sanitized and appropriate for the species. Fresh water (de-chlorinated for amphibian species) and nutritionally complete feed appropriate for the species. Hiding tubes, boxes, and perches of appropriate size.</p>	<p>Same as for group housed reptiles/other amphibians.</p>	<p>Food items and treats not to constitute a substantial portion of the standard diet.</p>
Xenopus	<p>Group housed in water tanks or tubs with PVC pipe or a floating device that is removable and easily sanitized. De-chlorinated water, temperature appropriate for species flushed daily post feeding, and free of significant debris. Nutritionally complete</p>	<p>Same as for group housed xenopus.</p>	



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

	commercially available food, appropriate for the species.		
Fish	Group housed in water tanks. De-chlorinated water, temperature appropriate for species continuous recirculation of water to maintain free of significant debris. Nutritionally complete commercially available food appropriate for the species.	Same as for group housed fish.	
Birds	Group housed whenever possible in smooth wire cages adequate for the species. Flight cages if possible or alternative enrichment made available. Perches adequate to prevent foot disease, water source available at all times, food nutritionally complete for the species, and access to materials for maintaining crop.	Same as for group housed birds.	

## Mice Cage Sizing and Density

### **POLICY REGARDING MICE CAGE SIZING AND DENSITY**

**PURPOSE:**

Liberty University seeks to provide adequate, humane housing for research animals stored and used in its facilities. As such, this policy outlines the appropriate measures that need to be taken to ensure that sufficient space is allocated for animals used in research. This policy follows the guidelines set forth in the *Guide for the Care and Use of Laboratory Animals*, 8<sup>th</sup> edition.

**POLICY:**

The principal investigator, his or her research staff, and if arranged, the vivarium manager, shall monitor animal housing density on a regular basis to allow proper weaning and adherence to the standards of this policy. If any deviations are noted, the PI must be notified, and deficiencies shall be corrected immediately. Failure to correct observed deficiencies may result in non-compliance.

**MINIMUM RECOMMENDED SPACE FOR MICE HOUSED IN GROUPS**

The following table uses recommendations from *The Guide* for rodent cages of the standard size (75in<sup>2</sup>). For smaller sized cages, consult the IACUC for the current recommendations.

**TABLE I. STANDARD CAGE SIZING**

<b>Animals</b>	<b>Weight (grams)</b>	<b>Floor Area of Cage per Animal (inches<sup>2</sup>)</b>	<b># of Adult mice per 75in<sup>2</sup> cage</b>
Mice in Groups	<10	6	12
	Up to 15	8	9
	Up to 25	12	6
	>25	≥15	5
Female + Litter		51	2 adults plus a single litter of any size

\*Standard density is 5 post-weaned mice per cage.

**HEAVY MICE**

If any mouse (breeder or experimental) weighs more than 45 grams, then that cage is restricted to four mice per cage.

**BREEDING MICE**

No more than two adults in a cage when a litter is born. Pups must be weaned by 21 days of age unless delayed weaning has been approved by the IACUC or attending veterinarian for health reasons. No more than one litter can be present in a cage, though exemptions can be made on a case-by-case basis by the IACUC for justifiable reasons (poor breeding, failure to thrive).

**POST-PARTUM BREEDING**

Removing the male from the harem post-breeding is highly recommended to avoid cage overcrowding. Advantages of this practice include:

- The male may be used in multiple harems (decreasing the total number of animals and sharing the genetics to a larger population)

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Decreased chance of cannibalism after birth
- Increase the number of pups that can be maintained in the same cage without exceeding density guidelines
- Prevent post-partum breeding of the females, which can result in the birth of a second litter before the first litter is weaned. *The Guide* prohibits multiple litters from the same female in the same cage at the same time.

### **SINGLE HOUSING**

Group housing is the standard established by Liberty University. For instances in which single housing is acceptable, see the policy regarding social housing.

### **OVERCROWDING**

To ensure that the institution remains within the confines of the established policy, the IACUC requires the following:

- Vivarium managers must track incidences of overcrowding, and report high frequency observations to the IACUC for consideration and reporting.
- The PI must meet with IACUC designees when 5 or more instances of overcrowding have occurred. A report of the meeting will be documented to ensure oversight and establish corrective action.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Non-Vivarium Animal Housing

### **POLICY REGARDING NON-VIVARIUM ANIMAL HOUSING**

#### **PURPOSE:**

To outline situations in which non-vivarium animal housing is appropriate, and when further approval is required for such arrangements.

#### **POLICY:**

Any housing or holding of laboratory animals in non-designated areas for a period of greater than 12 hours is strictly prohibited. Any deviation from this policy must be approved by both the attending veterinarian and the IACUC.

The transportation of any animal between designated housing facilities and temporary facilities must be performed in a manner appropriate for the species in question. For any questions, or for additional information, contact the attending veterinarian.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Quarantine and Stabilization of Research Animals

## **POLICY REGARDING QUARANTINE AND STABILIZATION OF RESEARCH ANIMALS**

### **PURPOSE:**

The purpose of this policy is to detail the quarantine and stabilization process for animals arriving to Liberty University from sources other than approved commercial vendors.

### **POLICY:**

The *Guide for the Care and Use of Laboratory Animals* states that “Quarantine is the separation of newly received animals from those already in the facility, in a way that prevents potential spread of contaminants, until the health and possibly the microbial status of the newly received animals have been determined.” (p. 110). *The Guide* also states that “regardless of whether the animals are quarantined, newly received animals should be given a period for physiologic, behavioral, and nutritional acclimation before their use”. (p. 111).

Animals arriving to Liberty University from sources other than an approved commercial vendor must undergo a quarantine period prior to their use. This protection is in place to minimize the potential for the introduction of disease into existing colonies.

### **PROCEDURE**

1. Inform the attending veterinarian that animals will be acquired from an outside source (non-commercial vendor or institution) so that he or she may establish an appropriate quarantine period, determine the potential risks, and if necessary, assign proper treatment.
  - a. In certain cases, if data from the source is sufficient to determine the health status of the animal(s), the attending veterinarian may bypass the quarantine process.
2. Regardless of quarantine status, all animals shall be given a minimum of 72 consecutive hours to acclimate to the new environment.
  - a. No major experimental procedures may be performed during this acclimation period, unless explicitly approved by the IACUC in the animal use protocol.
  - b. For larger animals, an acclimation period of 5 days may be appropriate, as determined by the attending veterinarian.

Failure to acclimate research animals may result in adverse effects on animal health and subsequently, research data.

Any faculty transferring animals from other institutions are required to consult the attending veterinarian and the IACUC.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Transportation of Research Animals

## **POLICY REGARDING TRANSPORTATION OF RESEARCH ANIMALS**

### **PURPOSE:**

This policy serves to outline the process for transporting laboratory animals both on and off of the Liberty University campus.

### **POLICY:**

*The Guide* (p. 107) states that:

- Animal transportation may be intra-institutional, inter-institutional, or between a commercial or non-commercial source and a research facility.
- The process of transportation must provide an appropriate level of animal biosecurity while minimizing zoonotic risks, protecting against environmental extremes, avoiding overcrowding, providing for the animals physical, physiologic, or behavior needs and comfort, and protecting the animals and personnel from physical trauma.
- Movement of animals within or between sites or institutions shall be planned and coordinated by responsible and well trained persons at the sending and receiving sites to minimize animal transit time or delays in receipt.

In order to comply with the above recommendations, Liberty University has adopted the following policy:

1. Any transportation of animals outside of an animal facility must:
  - a. Be approved by the IACUC or described in the approved IACUC protocol.
  - b. Be for a period of no longer than 12 hours (unless approved by the IACUC).
2. Appropriate vehicles for transporting animals must be used.
3. Animals must be transported in appropriate cages or carriers designated for each species.
4. Whenever possible, limit the exposure of the animals to public viewing by covering or concealing the animal cages during transport.
5. The following means of transportation are not permitted for transporting animals:
  - a. Public transit (shuttle bus, train, etc.)
  - b. Bicycles
  - c. Motorcycles
  - d. Motor-driven scooters
6. Transportation between animal facilities requires the approval of the facility managers, PIs, or designees.
7. This policy only extends to university owned research animals, and does not apply to privately owned animals, or animals being treated in a veterinary-client-patient relationship.
8. Any exemptions to this policy must be approved by the IACUC.

### **GUIDELINES ON VEHICULAR TRANSPORT**

- Animals must be transported in the passenger compartment only.
- The vehicle must provide adequate heating and cooling to maintain an appropriate environment for the animal(s).

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Crates/cages must be placed away from direct sunlight with adequate ventilation, and on non-porous material within the vehicle to allow for easy cleaning.
- Animals shall not be overcrowded at any time.
- Crates/cages must be secured properly in an upright manner, making sure that they are not tilted, rolled, or laid on their side.
- Drivers are to remain with or in the vehicle at all times when animals are present.
- The most direct route of transportation must be taken, i.e., door to door.
- Personnel must be aware of the potential for animal allergen exposure and risk to others that may be passengers in the vehicle.
- Take necessary steps to minimize stress during transport:
  - Handle cages gently, do not shake or drop
  - Low noise levels: keep windows closed, do not slam doors, keep radios low or off.
- Do not smoke in or around the animal transport vehicle or animal crates.

### **GUIDELINES ON ON-CAMPUS TRANSPORTATION**

- If using a vehicle, follow the above guidelines.
- If animals are to be transported on foot:
  - Mice, rats, and animals may be manually transported in secured cages
  - Cage lids must be secured, with low-residue adhesive tape or a bungee cord
  - Cages must be protected or covered from sunlight and visual observation by using an opaque drape or container.
  - If an underground connection is available between buildings, this is preferable to going outside.

## Instructional Protocol Specific

### Policies

#### Animal Use in Learning or Teaching

### **POLICY REGARDING ANIMAL USE IN LEARNING OR TEACHING**

#### **PURPOSE:**

This document serves to outline the use of animals in learning or teaching activities, and to what extent involved individuals need to be trained or approved to work with animals.

#### **POLICY:**

Any learning activity (teaching or demonstration) involving live vertebrate animals at Liberty University must be approved by the IACUC. Any such activity must be done in accordance with Liberty University policies and guidelines, the Animal Welfare Act, the Public Health Service Policy, and the Guide for the Care and Use of Laboratory Animals.

The Principal Investigator is responsible for ensuring that all participants (instructors, TAs, and students) using live vertebrate animals are listed on the protocol. The PI must also ensure that all participants are properly trained, informed, and aware of any regulatory requirements, hazards, or risks involved in the activity.

#### **CLASSIFICATIONS**

- Observers
  - May be in close proximity to animals during a learning activity, but do not handle the animals or perform any procedures
- Short-Term Participants/Students
  - Those participating in a learning activity involving 30 or fewer consecutive days of animal use or 15 or fewer total days of animal use over a six month period
- Long-Term Participants/Students
  - Those participating in a learning activity involving more than 30 consecutive days of animal use, or more than 15 total days of animal use over a six month period.

#### **REQUIREMENTS FOR THE ABOVE CLASSIFICATIONS**

- Observers
  - Not required to be listed on the animal use protocol; no CITI training is required
  - Not required to undergo mandatory training
  - Must be escorted by the PI, or a member of the PI's staff listed on the protocol
- Short-Term Participants/Students
  - Not required to be listed on the animal use protocol; no CITI training is required
  - PI must send a list of participants with the following information to the IACUC:
    - Associated with LU: Full name, Flame pass ID #
    - Unassociated with LU: Full name, title, affiliated institution
  - Must receive information on health risks and required PPE
  - Must undergo a training or orientation session administered by the PI
  - Must be supervised by the PI, or a member of the PI's staff listed on the protocol
- Long-Term Participants/Students



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Must be listed on the animal use protocol; CITI training is required
- Must undergo a training or orientation session administered by the PI
- Must be supervised by the PI, or a member of the PI's staff listed on the protocol

Miscellaneous

Policies

Inter-Institutional Animal Research

**POLICY REGARDING INTER-INSTITUTIONAL ANIMAL RESEARCH**

**PURPOSE:**

The Liberty University IACUC understands that investigators may, at times, perform animal research at offsite locations, or that other institutions may perform animal research on the Liberty University campus. This document provides a framework for decision making regarding the responsibility for the care and use of the animals.

**POLICY:**

Liberty University must adhere to the applicable laws and regulations regarding animal use outlined by the United States Department of Agriculture, or USDA, for species regulated by the Animal Welfare Act, and the Office of Laboratory Animal Welfare, or OLAW, for species regulated by the Public Health Service Policy. The status of any offsite facility may impact whether the Liberty University IACUC is required to obtain an inter-institutional Assurance, include the facility in the Liberty University IACUC Program Description, conduct semi-annual facility inspections, program reviews, or other oversight.

**USDA STATEMENT**

Assigns responsibility for offsite animals to the institution that owns the animals. If more than one institution is involved, responsibility for the animals being used is shared by the institution providing housing and care, and the institution that is involved in the planning and execution of the study. If an owning institution has a say in how offsite animals are used, then that institution has responsibility for the animals. Liberty University must ensure that the offsite facility is a registered research facility with the USDA, has an approved Veterinary Care Program in place, and has an IACUC which reviews the program of animal care and conducts facility inspections.

**OLAW STATEMENT**

Requires that the awardee institution be responsible for ensuring that all terms and conditions of the award, including the PHS animal welfare policy, are met. Jurisdiction in this case is based on the source of funding, not ownership. PHS Policy requires that all awardees and each performance site hold an approved Animal Welfare Assurance. OLAW negotiates Inter-Institutional Agreement Assurances of Compliance when an awardee institution without an animal care and use program or an IACUC will rely on the program of an Assured institution. Assured institutions have the option to amend their Assurance to cover non-assured performance sites, which effectively subjugates the performance site to the Assured institution and makes the Assured institution responsible for the performance site. If both institutions have full PHS Assurances, they may exercise discretion in determining which IACUC reviews protocols and under which institutional program the research will be performed. It is recommended that if an IACUC defers protocol review to another IACUC, then documentation of the review shall be maintained by both committees. Similarly, an IACUC needs to know about any significant questions or issues raised during a semi-annual program inspection by another IACUC of a facility housing a research activity for which that IACUC bears some responsibility or exposure.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

In many instances, such an agreement requires IACUC decision making on a case-by-case basis. In all cases, the Liberty University IACUC must adhere to all federal, state, local, and institutional regulations and policies.

**Post Approval Monitoring  
Guidelines**

**Congruence for NIH Funded Animal Studies**

**GUIDELINES ON CONGRUENCE FOR NIH FUNDED ANIMAL STUDIES**

**NIH REQUIREMENTS**

- The NIH Grants Policy Statement states that grantee organizations must establish appropriate policies and procedures to ensure the humane care and use of animals for PHS funded projects.
- As part of any NIH award, it is the responsibility of the institution to ensure that the proposed animal activities are congruent with corresponding IACUC approved protocols.

**OLAW DEFINITION OF CONGRUENCE**

- Congruence, as opposed to equivalence or approximation, is a relation which implies a kind of equivalence, though not complete equivalence.

**DETERMINING CONGRUENCE**

- The Liberty University IACUC has determined that the best method for determining congruence is for a direct, side-by-side comparison of the awarded grant and the IACUC protocol. This is consistent with OLAW recommendations for determining congruence.

**GRANT CONGRUENCE PROCEDURES FOR IACUC STUDIES**

- The reviewer must determine that the scope of work is similar for the grant and the protocol.
  - Aims and objectives must be congruent.
  - Ensure that research has not shifted from one area of emphasis to another.
- The reviewer must pay careful attention to the Research Strategy and Vertebrate Animal sections of the grant when comparing it to the protocol.
  - It is likely that the grant will be less detailed in its description due to page limits.
- OLAW maintains an understanding that research processes are often fluid, and as such allows PI discretion for study methodology, as long as it is within the scope of work.
- The reviewer must compare the animal species used, the animal numbers, and procedures proposed. OLAW does not expect the review to be “at the level of a microscope”.
  - Differences in animal numbers, procedures, test agents, doses, or routes, may not be incongruent, so long as the scope of the work is similar, e.g., injection vs. drug administration by gavage, substitution of one drug for a similar drug in a related class.
  - Addition of experimental groups, procedures, agents may not be incongruent, so long as the scope of work is similar, e.g., addition of a different behavioral test to measure neurological symptoms, implantation of a mini-osmotic pump instead of daily drug injections.
  - Changes in strains, such as genetically modified animals may not be incongruent, so long as the scope of work is similar, e.g., addition of a new KO strain in the same gene of interest.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Change in animal species may not be incongruent, so long as the scope of work is similar (e.g., KO mouse instead of wildtype rat).
  - However, change in animal species may often signal a change in scope and must be examined more critically.
- There is no expectation that standard veterinary drugs used for analgesia, anesthesia, or euthanasia must be identical, e.g., ketamine-xylazine is used instead of isoflurane.

### **WHAT TO DO IF A PROCEDURE LISTED IN THE GRANT IS NOT IN THE IACUC PROTOCOL:**

- The PI shall be asked for clarification. Explanations are documented in the protocol file.
- The PI may want to revise parts of the protocol to be consistent with the grant, or may want to inform NIH that procedures will not be conducted as originally proposed.

### **WHAT TO DO IF A PROCEDURE LISTED IN THE IACUC PROTOCOL IS NOT IN THE GRANT:**

- The PI shall be asked for clarification. Explanations are documented in the protocol file.
- The PI determines if there is a change in scope (as defined by the NIH), and if so, must notify the Grants Administration.
- Conversely, the PI must notify the IACUC of a change in scope as a result of NIH review.

### **WHAT TO DO IF A PROTOCOL AND GRANT ARE NOT CONGRUENT:**

- The non-congruent research or procedure is documented on the review sheet.
- The non-congruent research or procedure is listed on the approval letter, which is sent to NIH by the PI.

**Note: Investigators must perform research reviewed and approved by the IACUC, regardless of what is written in the grant.**

*The NIH relies on the IACUC to both review and approve animal procedure and ensure that the research is congruent with the scope of work approved by the NIH.*

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Policies

#### Non-Compliance

### **POLICY REGARDING NON-COMPLIANCE**

#### **PURPOSE:**

The Liberty University IACUC is responsible for the oversight of all active animal research or teaching protocols at the University. Part of this responsibility is to ensure compliance with all federal, state, and local laws. This document serves to outline instances of non-compliance, and indicate the extent of any follow-up actions.

#### **POLICY:**

Any issues or reports of non-compliance will be investigated by the IACUC, and any resulting work required to remedy the problem(s) may be charged to the PI. Such charges might include professional veterinary consultation, diagnostic charges, per diem, etc.

#### **NON-COMPLIANCE INCLUDES, BUT IS NOT LIMITED TO THE FOLLOWING:**

- Animal neglect or animal cruelty
- Conducting animal research that does not have an approved IACUC protocol
- Performance of procedures by personnel who are not listed on the approved protocol
- The use of anesthetics, analgesics, tranquilizers, antibiotics, or other medications that are not specifically listed on the approved protocol, are different from those listed on the protocol, or are not used in accordance with applicable law, protocol, or policy
- Survival surgery without the use of aseptic technique.\*
- Euthanasia procedures that differ from the approved protocol, or use of a method that has not been approved, or failure to use a method for ensuring death
- Lack of adequate training on the part of laboratory personnel for the procedures listed in the approved IACUC protocol
- Failure to provide or keep an accurate record of supporting documentation for animal care, post-op evaluations, or other study procedures
- Unsafe conditions for either the animals or laboratory personnel
- The use of outdated or expired materials, including drugs, experimental agents, suture, sterile supplies, etc.
- Animal overcrowding, or the failure to follow standard breeding practices
- Animal work conducted outside of approved facilities
- Animals are not acquired or transferred as stated in the approved protocol
- Housing and husbandry standards are not met

*\*Note: A dedicated surgical suite is not required for rodents and non-mammalian vertebrates. However, this is the most desirable option and shall be used if available.*

#### **PROCEDURES FOR ASSESSING AND HANDLING NON-COMPLIANCE ISSUES**

1. The IACUC office will notify the PI of the deficiency and document the issue to be reported to the IACUC and if applicable, the IO.
2. Generally, the compliance issue will be resolved with immediate corrective actions.
3. Depending on the severity of the compliance issue, it may be necessary to immediately contact the Attending Veterinarian or the IACUC office.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- a. Any non-compliance issue that represents a threat to the health and safety of the animals or laboratory personnel would require such notification, as would any substantial deviations from the approved protocol.
4. The IACUC Chair, in consultation with the Attending Veterinarian, will decide whether the non-compliance issue must be reported.

### **IACUC ACTIONS**

1. Require additional training for the PI and/or laboratory staff.
2. Require that the PI appear at the next IACUC meeting for discussion.
3. Require that the Attending Veterinarian take over animal care under the animal holding policy, where the PI would be responsible for any associated costs.
4. Temporarily suspend animal use privileges.
5. Notify the PI's department head, dean, and/or the IO.

### **SERIOUS OR UNRESOLVED ISSUES**

1. Are additional actions required to resolve the compliance issue(s)?
2. Will animal use privileges and protocol approvals be suspended or revoked?
3. Is additional reporting of follow-up reporting to OLAW, USDA, AAALAC, and any funding agencies necessary?

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Reporting of Animal Concerns

### **POLICY REGARDING THE REPORTING OF ANIMAL CONCERNS**

#### **PURPOSE:**

Liberty University is committed to protecting the welfare of all laboratory animals used in teaching and research. It is the responsibility of the IACUC to ensure that animals are being treated humanely and responsibly in accordance with Public Health Services Policy, the Animal Welfare Act, and the University's policies and guidelines. This document serves to provide information on how to report animal use concerns to the appropriate authorities.

#### **POLICY:**

All persons involved with the use of animals in research or teaching must be provided with adequate training on how to report concerns regarding animal care and use. Anyone may report an alleged incident, and under no circumstances will such reporting be detrimental to an individual's standing with the University. No person will be discriminated against or subject to any reprisal for reporting a concern or violation of any animal care policies or standards. The IACUC is required by law to investigate any alleged reports of animal abuse or mistreatment. If you observe or suspect animal abuse, mistreatment, or non-compliance with any federal, state, or local laws, or non-compliance with approved procedures, please report the incident. The IACUC will investigate all claims and will maintain confidentiality in all matters.

#### **METHODS FOR REPORTING CONCERNS**

Please use any of the following methods to report animal welfare concerns:

- **ANONYMOUS FORM AVAILABLE ON THE IACUC WEBSITE**  
[https://liberty.co1.qualtrics.com/jfe/form/SV\\_9norlIBTcjFgdp3](https://liberty.co1.qualtrics.com/jfe/form/SV_9norlIBTcjFgdp3)
- **LIBERTY UNIVERSITY IACUC**  
434-582-2827  
iacuc@liberty.edu
- **ATTENDING VETERINARIAN**
- **IACUC CHAIR**
- **THE RESPONSIBLE PRINCIPAL INVESTIGATOR (PI)**
- **THE LABORATORY MANAGER**



## Reporting to External Agencies

### **POLICY REGARDING REPORTING TO EXTERNAL AGENCIES**

#### **PURPOSE:**

This policy serves to outline the process and guidelines for reporting and responding to external agencies, including OLAW, PHS, and the media.

#### **POLICY:**

The following policy outlines the necessary steps that must be taken in order to report to the appropriate federal agencies, or to the media.

#### **REPORTING TO NIH/OLAW AND APHIS/AC**

The IO is solely responsible for reporting to and interacting with NIH/OLAW and APHIS/AC in order to avoid any inadvertent errors or misinformation regarding either party. The following concerns will be reported to OLAW by the IO, or his or her designee, upon being informed by the IACUC:

- Non-compliance with IACUC-approved protocols
- Initiating unapproved animal work
- Deviation from the approved Liberty assurance of compliance with PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy)
- Animal activities conducted by unauthorized or unqualified individuals
- Ongoing, unresolved problems in animal care and use
- Shortcomings in the animal care program that jeopardize the health and well-being of animals or cause their death
- The IACUC's suspension of any animal activities by the IACUC (whether temporary or permanent).

While not prohibited, it is strongly discouraged that any individuals, research staff, or principal investigators (PIs) reach out to the federal agencies without first notifying both the Research Ethics Office and the Office of the Provost. All contacts, questions, or reports should flow through the Research Ethics Office or the Office of the Provost. Individuals are strongly encouraged to contact the Research Ethics Office regarding with regard to any questions, comments, or concerns relating to animal welfare or use before engaging other agencies.

#### **REPORTING TO THE MEDIA OR OTHER NEWS AGENCIES**

If a PI or other member of the research staff is contacted by an external news agency, direct the agency to the Liberty University External Communications Office, 434-582-7751. The External Communications Office will be able to respond properly to any questions regarding IACUC protocols or animal research at Liberty University. The IACUC must be promptly notified of any requests for public comment from external news agencies.

Under no circumstances shall unauthorized individuals be allowed entry into the university's animal housing facilities. Further, all persons involved with animal research shall refrain from any photography or video recording unless it is explicitly approved by the IACUC. Under no circumstances should individuals post to social media any accounts, images, or videos of animal procedures, housing, or other associated activities.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Review of Animal Welfare Concerns

## **POLICY REGARDING THE REVIEW OF ANIMAL WELFARE CONCERNS**

### **PURPOSE:**

This policy serves to outline the process by which the Liberty University IACUC reviews and responds to reported concerns of animal welfare or protocol deficiencies.

### **POLICY:**

The 8th edition of the Guide for the Care and Use of Laboratory Animals lists among the IACUC's oversight functions the "establishment of a mechanism for receipt and review of concerns involving the care and use of animals at the institution."

Any alleged reports of animal abuse or mistreatment shall be investigated by the LU IACUC. All claims will be kept confidential, and any persons making such a report will be free to do so without fear or reprisal. Reports may also be made anonymously.

### **REGULATIONS ESTABLISHING AUTHORITY TO REVIEW WELFARE CONCERNS**

The regulations issued pursuant to the Animal Welfare Act [9 CFR §2.31(c)(4)] and the Public Health Service Policy, Section IV.B.4, require that:

The IACUC review, and, if warranted, investigate concerns involving the care and use of animals at the research facility that result from received public complaints or reports of noncompliance from laboratory or research facility personnel or employees.

In accordance with the AWA, [(9 CFR Ch. 1), Part 2-Subpart C, 2.32 (b),(c)(4)]:

Training and instruction shall be made available as to how deficiencies in animal care and use can be reported by any employee of the facility. No facility employee, committee member or laboratory personnel shall be discriminated against or subject to any reprisal for reporting violations of any regulation or standards under the Act.

### **MECHANISMS FOR REPORTING**

- Reporting Posters in Animal Use Facilities
  - Prominently displayed in each applicable animal facility;
  - Distributed to investigators' laboratories during the semi-annual facility inspection so that it may be re-posted at that time;
  - Distributed at laboratory training sessions to all those involved with animal use in teaching or research;
  - Distributed during workshops, seminars, and other training sessions regarding laws, regulations, policies, and procedures involving animal care, treatment and use.
- The Liberty University IACUC Website
  - Contains a link to an anonymous reporting form
  - Contains contact information for reporting to the IACUC, IACUC Chair, Attending Veterinarian, or the IACUC Office

### **REPORTING CONCERNS**

- Anonymously
  - a. The IACUC Website
- Directly

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- a. The IACUC Chair
- b. The IACUC Office
- c. The Attending Veterinarian
- d. Responsible Principal Investigator(s)
- e. Overseeing Laboratory Manager(s)

*Note: The above concerns may be reported in person, via telephone, via e-mail, or in writing.*

### **IACUC HANDLING OF CONCERNS**

- 1) Individuals shall be free to report concerns related to animal care, treatment, and use at Liberty University without fear of reprisal.
- 2) Any reported concerns will be forwarded in writing to the IACUC chair and the IO (if they have not already been advised).
- 3) The IACUC chair will convene the committee and present the problem for discussion.
- 4) IACUC will review the matter and make recommendations that will be conveyed to the IO and the attending veterinarian.
- 5) Depending on the nature of the concern, the IACUC chair, the attending veterinarian, and/or the IO will take appropriate action(s).
  - a. The above persons will ensure that there is no reprisal against the individual reporting the concern.

Protocols  
Guidelines

Performing a Literature Search for Alternatives

**GUIDELINES ON PERFORMING A LITERATURE SEARCH FOR  
ALTERNATIVES**

**PURPOSE:**

The requirement to perform a literature search for alternatives is based on the concept of the three R's. The three R's are **reduction** (in the number of animals used), **refinement** (of techniques and procedures to reduce pain or distress), and **replacement** (of animals with non-animal techniques, lesser species, or computer modeling). This document serves to outline a strategy for performing research that is consistent with the three R's.

**GUIDELINES:**

A strategy that has been suggested includes performing the research on alternatives in two separate phases:

- **PHASE 1: CONSIDERATION OF REDUCTION AND REFINEMENT**
  - Look for studies that relate to the following:
    - No unnecessary duplication
    - Appropriate animal numbers
    - Best pain relieving agents (anesthesia and analgesics)
    - Other methods of reducing pain and/or distress
  - Read the materials and methods sections
  - In Phase 1 of the literature search, it is suggested that the word *alternative* not be used, as this can lead to limited results. In certain disciplines, such as toxicology or education, using the term *alternative* would be acceptable due to the high number of cases where alternatives have been previously developed
  - Suggested search terms: analgesic, painkiller, anesthesia, sedative, housing, facility management, caging, welfare, well-being, pain, distress, technique, procedure, method, assay
- **PHASE 2: CONSIDERATION OF REPLACEMENT**
  - Look for studies that relate to the following:
    - Animal and non-animal models as alternatives
  - The word *alternative* is suggested for these searches, as well as the word *model*, as these will help to narrow the search to more applicable studies
  - Suggested search terms: vitro, culture, simulation, artificial, tissue, cell, organ, model, virtual

Suggested databases include (but are not limited to):

- EMBASE
- TOXLINE
- Web of Science
- PubMed

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- Zoological Record
- AGRICOLA (USDA)
- PsycINFO

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Policies

#### Administrative Review of Protocol Amendments

## **POLICY REGARDING ADMINISTRATIVE REVIEW OF PROTOCOL AMENDMENTS**

### **PURPOSE:**

This document serves to outline instances in which the IACUC may administratively issue amendments to existing IACUC-approved animal use protocols.

### **POLICY:**

According to the NIH/OLAW Notice NOT-OD-03-046, it is stated that “IACUCs may, by institutional policy, classify certain proposed additions or changes in personnel, other than Principal Investigator, as ‘minor’ provided an appropriate administrative mechanism is in place to ensure that all such personnel are appropriately identified, adequately trained and qualified, enrolled in applicable occupational health and safety programs, and meet other criteria as required by the IACUC.”

Given the above statement, the Liberty University IACUC has established that certain minor changes may be approved administratively, without the need for Full Committee Review or Designated Member Review. Any such changes must be done so using the Protocol Amendment Form. Completed forms shall be submitted to [iacuc@liberty.edu](mailto:iacuc@liberty.edu).

### **CHANGES THAT MAY BE APPROVED ADMINISTRATIVELY INCLUDE:**

- Changes in personnel other than the Principal Investigator (PI)
- Changes in previously approved animal numbers as a result of technical, vivarium, or vendor related issues
  - Additions are limited to  $\leq 10\%$  of the originally approved number
- Changes in procedures that occur after euthanasia

### **THE FOLLOWING PERSONS ARE QUALIFIED TO MAKE ADMINISTRATIVE APPROVALS:**

- IACUC Chair
- IACUC Administrator
- Attending Veterinarian
- An IACUC Member designated by the IACUC Chair

### **ACCORDING TO NIH/OLAW, CHANGES THAT DO NOT QUALIFY FOR ADMINISTRATIVE APPROVAL INCLUDE\*:**

- Changes in study objectives
- Changes in the degree of invasiveness of a procedure or discomfort to an animal
- Changes in species or in approximate number of animals used
- Changes in anesthetic agents
- Use of withholding of analgesics or methods of euthanasia
- Changes in duration, frequency, or number of procedures performed on an animal

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Amendments Requiring a New Protocol

### **POLICY REGARDING AMENDMENTS REQUIRING A NEW PROTOCOL**

#### **PURPOSE:**

This policy serves to identify which protocol amendments require a new protocol application prior to being processed and reviewed by the IACUC.

#### **POLICY:**

The Liberty University IACUC requires that the following amendments be submitted with a new protocol application prior to being reviewed by the committee:

- Change in the purpose, aim, or objectives of a study
- Change in the Principal Investigator (PI)
- Change or addition of a new species
- Large increases in animal numbers (>100% of originally approved number)
- Repeating an experiment with additional animals

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Closure of Active IACUC Protocols by PI Request or Expiration

### **POLICY REGARDING THE CLOSURE OF ACTIVE IACUC PROTOCOLS BY PI REQUEST OR EXPIRATION**

#### **PURPOSE:**

This policy provides guidance on the process of closing an active IACUC research protocol, either by expiration or voluntarily by PI request (no more animal work will be performed).

#### **POLICY:**

Protocols must be closed out properly at the conclusion of their three-year approval period or at the conclusion of all animal work. Unless a protocol has been previously closed out, it will be considered closed upon the third anniversary of its approval date. Researchers who wish to continue protocols beyond this initial three-year period must submit a new protocol to the IACUC for approval. Protocols must be closed by notifying the IACUC and other appropriate parties, whether the decision is voluntary or not.

#### **RIGHTS**

The PI has the right to close out an IACUC protocol at any time during the period in which it is approved. If a protocol is voluntarily closed, but a PI wishes to restart animal work, a new protocol must be submitted to the IACUC for approval. All protocol closeouts must be consistent with applicable laws and policies. Failure on the part of a PI to adequately close out a protocol may result in non-compliance.

#### **PROCESS & GUIDELINES**

The process of closing a protocol allows the IACUC to fulfill its regulatory obligations to the federal agencies. Following the procedures for closing a protocol ensures that:

- The protocol has been conducted in accordance with the approved protocol
- Modifications received IACUC approval prior to implementation
- No unanticipated or adverse events were experienced, or such events were documented

Being that the process of closing out an IACUC protocol is a federally mandated activity, the following guidelines are in place:

- 90 days prior to protocol expiration, a letter will be sent to the Principal Investigator notifying them of the upcoming expiration.
- 45 days prior to protocol expiration, a notification will be sent to the PI alerting them of the impending annual review.
  - If a protocol is closed outside of its anniversary date, an annual review form will be sent to the PI. This form must be filled out and returned within 30 days of the protocol closing.
- The annual review reports will be reviewed appropriately. Any protocols with potential compliance issues will be further investigated by the IACUC.



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Eligibility and Responsibilities of the Principal Investigator

### **POLICY REGARDING ELIGIBILITY AND RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR**

#### **PURPOSE:**

This document serves to outline the eligibility requirements for serving as a Principal Investigator on a Liberty University animal use protocol.

#### **POLICY:**

Serving as the PI entitles the investigator to certain rights and responsibilities.

#### **ELIGIBILITY**

In order to be eligible to serve as PI for an IACUC approved protocol, the person must be available to devote sufficient time and attention to the study to ensure proper conduct. In most cases, a Principal Investigator on an IACUC protocol will be a full-time member of Liberty University faculty or a senior research appointee. This may include the following titles:

- Professor/Clinical Professor
- Associate Professor/Clinical Associate Professor
- Assistant Professor/Clinical Assistant Professor
- University Professor
- Senior Scientist
- Research Scientist
- Principal Research Scientist
- Veterinarians

The IACUC will also consider the following roles as an approved PI on protocols primarily related to animal use in teaching and demonstration that will be no higher than USDA Pain Category C:

- Instructor
- Lecturer/Senior Lecturer
- Post-Doctoral Student (Supervising faculty must be listed as a co-investigator)
- Research Associate

The following will not ordinarily be allowed to serve as a PI on an animal use protocol; however, the IACUC will consider these requests on a case by case basis:

- Emeritus faculty
- Visiting/Adjunct faculty
- Courtesy or other appointments
- Laboratory Technicians

While not eligible to serve as a Principal Investigator on animal use protocols, Liberty University students may be listed as co-researchers.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### **RESPONSIBILITIES**

The PI has primary responsibility for all aspects of the protection of animal species used in research, including compliance with all Federal and University policies and procedures. Additionally, it is the sole responsibility of the PI to ensure that all research involved in a PI's project also comply with said regulations, policies, procedures, and guidelines. The PI must also make sure that no research staff or consultants perform research tasks if there is likely to be a conflict of interest, unless the conflict has been previously reported to the IACUC and proper safeguards have been approved.

### **RIGHTS**

- Applications shall be reviewed by the IACUC in accordance with the ethical principles described in the Animal Welfare Act and Public Health Service Policy on Humane Care and Use.
- When protocols are submitted, the IACUC shall review the applications as specified in the policy, barring any unforeseen and/or insurmountable problems.
- All decisions of the IACUC shall be conveyed to the PI in writing (electronically or otherwise).
- The PI may consult with the IACUC Coordinator, Chair, or designee if the PI is unclear about the rationale for its decisions or if any questions arise at any time related to the application or approved protocol.

### **RESPONSIBILITIES OF THE PI UPON LEAVING THE UNIVERSITY**

When a PI plans to leave the university and continue the research activities at another institution, he or she must notify the IACUC in writing. This will allow the IACUC to close the active research file. The PI is responsible for obtaining IACUC approval at the new institution. If the research project will continue at Liberty University, the PI must submit a completed Change of PI form to the LU IACUC office. Otherwise, the protocol must be closed in a manner consistent with the approved method and LU policy.

In instances where the PI has departed the University without closing or transferring the protocol, refer to the "Policy Regarding Protocol Closure or Transfer in the Event of a Principal Investigator's Absence" for further guidance.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Exempt Animal Protocols

### **POLICY REGARDING EXEMPT ANIMAL PROTOCOLS**

#### **PURPOSE:**

The purpose of this policy is to provide guidance on when animal activities may be exempt from IACUC oversight, veterinary authority, or facility inspections.

#### **POLICY:**

In all cases, the IACUC should be notified of any proposed animal activities, in writing, via the IACUC Animal Exemption Request Form. The IACUC is solely responsible for making any final determinations as to the exemption status of proposed animal activities at Liberty University.

#### **FIELD STUDIES**

Field studies that are observational in nature are exempted from completing an IACUC application and receiving IACUC approval, provided the proposed use does not involve any of the following:

- Capturing
- Trapping
- Chemical restraint
- Physical restraint
- Invasive procedures
- Any form of direct or indirect manipulation

#### **STUDIES THAT DO NOT INVOLVE THE USE OF LIVE ANIMALS**

Any research or teaching protocols that **do not** involve the care or use of live animals (i.e., only involve the use of cadavers, body parts, tissues, blood, etc.) may qualify for an exemption provided that the animal cadavers, body parts, tissues, blood, etc. are obtained from an IACUC approved source and are disposed of in accordance with state law and LU policies for disposal.

#### *Approved Sources*

- United States Department of Agriculture (USDA) licensed dealers
- USDA approved/inspected slaughterhouses
- Animals from the State of Virginia presented for euthanasia and/or necropsy that would otherwise be disposed of as pathological waste
- Veterinary hospital patients that die or are euthanized that would otherwise be disposed of as pathological waste with the consent of the owner
- Local pound/shelter (i.e., derived from stray/unclaimed animals that are euthanized in accordance with USDA regulations, Virginia statutes, and municipal ordinances)
- An IACUC approved animal use protocol (i.e., derived from dead animals that would otherwise be disposed of as pathological waste). **Note:** An IACUC approved protocol is required if additional/special ante-mortem procedures are involved in the harvesting of tissue, blood, etc.)
- Archival tissues from tissue banks or museum collections
- Commercial grocery store and/or meat market/producer (i.e., fresh/frozen beef, pork, poultry, fish, etc.)

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### *Other Sources*

Investigators performing research, teaching, or testing that involves the use of animal cadavers, body parts, tissues, blood, etc. from a source not listed above must explicitly detail the following in the exemption request:

- PI name, office, department, phone number, email address;
- A brief description of what the animal cadavers, body parts, tissues, etc. will be used for;
- The source(s) of animal cadavers, body parts, tissues, blood, etc.;
- Where the animal cadavers, body parts, tissues, etc. will be stored and/or used;
- How the animal cadavers, body parts, tissues, blood, etc. will be disposed of

### **INVERTEBRATE ANIMALS**

The Liberty University IACUC does not typically require approval for research or teaching involving the use of invertebrate animals. That said, exemption requests for such activities must be made through the IACUC. In all instances, animals shall be treated in an ethical manner, using sound, justifiable scientific practices.

Genetically Modified Animals

**POLICY REGARDING GENETICALLY MODIFIED ANIMALS**

**PURPOSE:**

This policy serves to establish specific considerations that must be made in animal use protocols involving genetically modified animals (GMAs). This applies to all instances involving genetically modified animals, including transgenic or targeted mutations created to study gene expression. The Guide states: “With their inherent potential for unanticipated phenotypes, GMAs are an example of models for which increased monitoring for unexpected outcomes could be implemented.”

**POLICY:**

Protocols that utilize genetically modified animals must consider the following criteria when designing protocols:

- The effect that the genetic modification will have on the health of the animal must be described in detail. If this is a new modification and the health effects are unknown, describe the likely health outcomes of the genetic change according to current understanding of the gene in question and the disease state under study.
- Endpoints to address potential or known adverse health effects:
  - The PI must provide general humane endpoints in case a severe debilitating phenotype develops and should provide the IACUC with this information in writing when the new mutant has been developed or at the next annual review of the protocol.
  - Endpoints are relevant both in the context of experimental procedures and with regard to the potential pain or distress that is caused by the genetic modification itself.
  - The protocol should include endpoints for the initial phase of the study when the phenotype of new genetic modification is being characterized, as well as for later phases of the study for both experimental and non-experimental (i.e. breeding) animals.
- Animal number estimates for each phenotype
- Institutional Biosafety Committee considerations, including:
  - Function of the gene being inserted, or the biological effect of the knock-out gene
  - Risks associated with the GMA that may create a greater hazard for personnel
  - If GMAs are being bred, what is the expected biological outcome of the novel strain

Any newly developed genetically modified animals that have not previously been characterized must be closely scrutinized for general health and behavioral abnormalities that may impact the animal’s well-being.

Many GMA’s have already been developed and characterized. For animals with a known phenotype, a maximum holding period should be set to avoid the development of known or predictable problems in strains that have debilitating phenotypes.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### IACUC Protocol Submission

## **POLICY REGARDING IACUC PROTOCOL SUBMISSION**

### **PURPOSE:**

The Liberty University Institutional Animal Care and Use Committee requires that all research and teaching involving the use of vertebrate animals have an approved IACUC protocol. This policy outlines the process of applying for such approval.

### **POLICY:**

Approved protocols assure appropriate care and use of the animals, as well as the safety of the research personnel. The Liberty University IACUC reviews protocols to ensure compliance with all federal, state, and local laws. Any research or teaching project involving vertebrate animals must have approval prior to its starting, regardless of funding source.

Any proposed animal use must be consistent with the *Animal Welfare Act Regulations*, the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, and the *Guide for the Care and Use of Laboratory Animals (The Guide)* unless other justification has been provided and approved.

All necessary forms are available on the Liberty University IACUC website.

### **VETERINARY REVIEW**

All proposed studies are subject to pre-review by the Attending Veterinarian.

### **POST APPROVAL**

Once approved, protocols will be valid for a period of three years. Approved IACUC protocols must be renewed every year, and will require resubmission if the study extends beyond the initial three-year period.

### **EXEMPTIONS**

Certain studies are exempt from needing an IACUC protocol. These include:

- Protocols involving the use of invertebrate animals.
- Cadaver use in non-AWA regulated animals that have not been manipulated for research prior to acquisition.
- Studies involving the use of animal tissues received from a slaughterhouse. (You must notify the IACUC of the possession of these animal tissues, per IACUC policy).
- Observational studies in which no direct contact or manipulation of the animal, its diet, or its environment is made.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Procurement of Laboratory Animals

## **POLICY REGARDING PROCUREMENT OF LABORATORY ANIMALS**

### **PURPOSE:**

This policy is designed to provide uniform procedures for the procurement and purchasing of laboratory animals at Liberty University.

### **POLICY:**

The *Guide for the Care and Use of Laboratory Animals—8<sup>th</sup> Edition* states:

“All animals must be acquired lawfully, and the receiving institution should ensure that all procedures involving animal procurement are conducted in a lawful manner. Before procuring animals, the principal investigator should confirm that there are sufficient facilities and expertise to house and manage the species being acquired. Procurement of animals should only be done upon receipt of IACUC approval.”

“The use of purpose-bred and preconditioned animals is therefore preferable when consistent with the research, teaching, and testing objectives. In general, animals used for scientific purposes should not be obtained from pet stores or pet distributors due to the unknown or uncontrolled background of animals from these sources and the potential for introducing health risks to personnel and other facility animals. Breeding colonies should be established based on need and managed according to principles of animal reduction such as cryopreservation for rodent stocks or strains.”

Whenever possible, acquire animals from one of the following approved vendors:

**TABLE I. LIBERTY UNIVERSITY APPROVED ANIMAL VENDOR LIST**

<b>Rats and Mice</b>	Harlan, Charles River, Taconic Farms, Jackson Labs, Hilltop Lab Animals
<b>Fish</b>	Carolina Biologicals

### **ANIMALS PROCURED FROM OTHER VENDORS**

Consult the IACUC prior to purchasing animals if any of the approved vendors are not available.

### **ANIMALS TRANSFERRED FROM OTHER INSTITUTIONS**

If you are receiving animals from another institution, or are a new PI transferring animals to LU, such animals may be subject to quarantine or holding prior to being permanently housed at the university. For more information, see the quarantine policy.

### **CONSIDERATIONS WHEN ORDERING**

The PI is responsible for determining appropriate delivery dates and ordering schedules. The PI must ensure that appropriate resources are available for receiving the animals on the designated delivery dates.

Orders should be placed using the request form on the IACUC website. For information on ordering, visit the animal ordering page on the IACUC website:

- <http://www.liberty.edu/academics/graduate/iacuc/index.cfm?PID=34762>

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

Use of DMR Subsequent to FCR for Protocol Modification Requests

### **POLICY REGARDING THE USE OF DMR SUBSEQUENT TO FCR FOR PROTOCOL MODIFICATION REQUESTS**

#### **PURPOSE:**

The purpose of this policy is to establish conditions under which Designated Member Review (DMR) may be used for verifying protocol modification requests after Full Committee Review (FCR). The following policy is advised by NOT-OD-09-035, released January 8<sup>th</sup>, 2009.

#### **POLICY:**

When the Full Committee requests modifications to a protocol submission prior to receiving approval, the committee may take the following actions regarding the use of DMR:

1. All IACUC Members are in Attendance at a convened FCR
  - a. If all IACUC members are in attendance at the meeting, the committee may vote to require modifications to secure approval and have the revised protocol reviewed by DMR, or by FCR.
2. All IACUC Members are not in Attendance at a convened FCR
  - a. Per this policy, a quorum of members present at a convened meeting may decide by unanimous vote to use DMR subsequent to FCR when modifications are 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.
  - b. One or more designated reviewers shall be assigned by the Chair, and must conduct DMR as outlined in the IACUC Handbook.
    - i. All reviewers must be unanimous in their decisions, otherwise the protocol shall be returned to the full committee for continued review.



# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Use of Literature Searches for Alternatives

## **POLICY REGARDING THE USE OF LITERATURE SEARCHES FOR ALTERNATIVES**

### **PURPOSE:**

Federal regulations require those requesting IACUC approval for protocols involving USDA-covered species to perform a literature search for alternative methods. This document establishes the requirement for Liberty University IACUC protocols.

### **POLICY:**

In applying for IACUC approval, the principal investigator must provide the committee a written narrative description of the methods and sources used to determine that alternatives are not available. The description and narrative of the literature search is described in the PI's answer to question 7 on the Liberty University Application for the Use of Animals in Research.

### **USDA-COVERED SPECIES DEFINITION**

Any live or dead dog, cat, non-human primate, guinea pig, hamster, rabbit, or any other warm-blooded animal, which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet. This term excludes birds, rats of the genus *Rattus*, and mice of the genus *Mus* bred for use in research; horses not used for research purposes; and other farm animals, such as, but not limited to, livestock or poultry used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.

### **REQUIREMENTS**

1. The PI must consider alternatives to any procedures that may cause more than momentary or slight pain or distress to the animals. The PI must provide a written narrative of the methods and sources used to determine the availability of alternatives, including any refinements, reductions, or replacements.
2. The literature search for alternatives must be performed and completed at the time of a new protocol application. This criteria includes any three-year renewals.
3. A minimum of two appropriate databases must be searched, and the search results must be provided along with the descriptive narrative in the protocol application.
4. Any literature that the PI is the author or co-author of shall not be used as justification for any animal use or procedure, unless no other literature exists, or information on the topic is limited.
5. The PI shall include the most recent literature available on the topic to ensure that best practices are maintained, and appropriate methodology is used.
6. The PI must keep a record of the search results and narrative for three years after completion of the study. This record must be produced if requested by inspectors.

For assistance with completing a literature search, refer to the guidelines on performing a literature search for alternatives or contact the IACUC.

## Training & Personnel

### Guidelines

#### Health and Safety

### **GUIDELINES REGARDING HEALTH AND SAFETY**

#### **PURPOSE:**

The Liberty University IACUC is dedicated to the health and safety of all laboratory personnel working on approved IACUC protocols. Working with or around animals and their waste products can expose personnel to health and safety risks. The primary risk identified by the IACUC is the development or worsening of animal allergies. Another risk to be aware of is the possibility of zoonotic disease transmission; however, this is not as likely.

#### **GUIDELINES:**

Prior to starting work with animals in teaching or laboratory situations, it is recommended that personnel are properly evaluated by their primary care provider to determine the need for additional protective measures.

Allergy symptoms may include any or all of the following: sneezing, runny or stuffy nose, itchy or water eyes, cough, shortness of breath, or skin rash. **If at any time you suspect that you may be experiencing an allergy or other health effect related to animal exposure, contact emergency services or seek advice from your primary care provider (depending on the severity of the situation).**

#### **SAFE LABORATORY PRACTICES**

- Wear the necessary Personal Protective Equipment (PPE), including:
  - Gloves
  - Lab Coat
  - Safety Glasses
- Know how to properly handle the species being used
- Observe and obey all laboratory signs and warnings
- Wash any animal bite or scratch immediately with disinfectant soap and notify the laboratory supervisor
- Seek proper medical attention following any injury or incident
- Practice good hygiene by washing hands frequently when in contact with animals
- Do not eat, chew gum, drink, store food, apply cosmetics, or smoke in any laboratory or animal facility

## Short Term Researcher Training

### **GUIDELINES REGARDING SHORT TERM RESEARCHER TRAINING**

#### **PURPOSE:**

Liberty University is required by federal law to ensure that all individuals working with animals are appropriately trained in the proper care and use of laboratory animals. This document provides a general overview of training topics that short term researchers should be exposed to prior to starting work with animals at Liberty University.

#### **GUIDELINES:**

Short term researchers are required to receive animal and health awareness training prior to beginning work on an IACUC protocol. While CITI training is not a requirement for short term researchers, the PI must provide adequate training for short term researchers in the areas listed below, at minimum. This sheet provides a general overview and is not intended to be an all-encompassing training resource. Short term researchers do not have to be listed on the IACUC protocol, however, a list of participants must be provided to the IACUC.

#### **ANIMAL WELFARE**

Animal use must be limited to research that is deemed necessary, which benefits humans and/or animals. All animals must be treated humanely. Animal use at LU is a privilege, not a right, and as such, this privilege can be revoked.

#### **THE 3 R'S OF ANIMAL RESEARCH**

*Replacement*: replace animals with non-animal models, cell cultures, or computer modeling wherever possible. *Reduction*: reduce the number of animals used by using statistical methods to determine the necessary number and prevent duplication. *Refinement*: consistently update procedures to minimize pain and distress, improve analgesics, and use varied approaches to surgical methods.

#### **ANIMAL RESEARCH REGULATIONS AND GUIDELINES**

The following regulations are in place regarding the care and use of animals in research and teaching: The Animal Welfare Act (AWA), Public Health Service (PHS) Policy, State and local laws, and University policy.

#### **ANIMAL RESEARCH “BEST PRACTICES”**

The following provide best practices on procedures and care of research animals: The Guide for the Care and Use of Laboratory Animals, the American Veterinary Medical Association (AVMA), and the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

#### **THE IACUC AND ITS PURPOSE**

The IACUC is responsible for the following: reviewing and approving submitted animal care and use protocols, providing semi-annual reviews and inspections of the animal facilities and program, investigating any concerns regarding animal care or abuse, and reporting to the Institutional Official.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### **RISKS AND HAZARDS OF WORKING WITH LABORATORY ANIMALS**

Risks and hazards include: scratch, bite, needle stick, or injuries from animals. Allergies may also result from working with animals (dander, urine, etc.). Potential for zoonotic disease transmission also exists and must be considered when working with animals. If you are exposed to any of the above while working with animals, report to the laboratory supervisor and submit an incident report.

### **PERSONAL PROTECTIVE EQUIPMENT (PPE)**

The use of PPE is required when working with animals. Its purpose is to protect researchers from the animals and animals from the researcher. Based on personal health status or the status of the animal, additional PPE precautions may be required. Further, proper use of PPE prevents the transmission of disease from animal to animal.

### **ANIMAL WELFARE CONCERNS AND REPORTING**

Liberty University is dedicated to the humane care and use of animals in all of its facilities. By law, the IACUC must investigate all claims of animal abuse or neglect. Anyone can report animal care and use concerns without fear of retribution. More information is available on the Liberty University IACUC website.

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Policies

Training of Principal Investigators, Researchers, and Animal Care Staff

### **POLICY REGARDING THE TRAINING OF PRINCIPAL INVESTIGATORS, RESEARCHERS, AND ANIMAL CARE STAFF**

#### **PURPOSE:**

This policy has been established as a part of Liberty University's commitment to providing adequate training for animal researchers including Principal Investigators, Course or Lab Instructors, Teaching or Research Staff, and Animal Care Staff.

#### **POLICY:**

The Animal Welfare Act Regulations Sec. 2.32 (a) and (b) specify: *"It shall be the responsibility of the research facility to ensure that all scientists, research technicians, and other personnel involved in animal care, treatment, and use are qualified to perform their duties. This responsibility shall be fulfilled in part through the provision of training and instruction to those personnel. Training and instruction shall be made available, and the qualifications of personnel reviewed, with sufficient frequency to fulfill the research facilities responsibilities..."*

PHS Policy Sec. IV.C.1.f places the responsibility specifically with the IACUC to ensure that personnel conducting procedures on research animals are appropriately qualified and trained in those procedures.

The Animal Welfare Act Regulations Sec. 2.32 (c) require that training and instruction of personnel must include guidance in at least the following areas:

1. Humane methods of animal maintenance and experimentation:
  - a. Basic needs of each species of animal used
  - b. Proper handling and care for each species of animal used by the facility
  - c. Proper pre-procedural and post-procedural care of animals
  - d. Aseptic surgical methods and procedures
2. Concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress
3. Proper use of anesthetics, analgesics, and tranquilizers for any species used in the facility
4. Methods for reporting deficiencies in animal care, including deficiencies reported by any employee of the facility. No facility employee or committee member will face reprisal for reporting violations of any regulation of standards under the AWA.
5. Utilization of services available for acquiring information (National Library of Medicine, etc.) on the following topics:
  - a. Appropriate methods of animal use and care
  - b. Alternatives to the use of live animals in research
  - c. Methods that could prevent unintended and unnecessary duplication of research involving animals

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

- d. The intent and requirements of the AWA.

### **TRAINING REQUIREMENTS**

In order to ensure that Liberty University researchers (students, staff, fellows, and faculty) receive orientation to Federal and University policies regarding animal research, those involved in research or teaching with vertebrate animals at LU are required to participate in CITI training. CITI training completion must be verified by the Research Ethics Office prior to individuals engaging in research or teaching activities involving the use of animals. The Institutional Animal Care and Use Committee (IACUC) may also require additional training for each individual, depending on their prior training and experience with animals or the nature of the protocol.

Animal researcher training is required of all protocol personnel once every three years. CITI training courses may require that training be completed on a more frequent basis, depending on the nature of the course.

It is the responsibility of the principal investigator (PI) to ensure that all animal users complete the required IACUC training, as well as any lab, species, or procedure specific training. This training should include daily animal husbandry and recordkeeping requirements.

### **PRIOR TO WORKING WITH ANIMALS, RESEARCHERS MUST COMPLETE:**

CITI Training

Research Occupational Health Program (ROHP) Training & Enrollment

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

### Approval and Revision History

<b>ANIMAL CARE AND USE</b>	<b>Initial</b>	<b>Revised</b>
Anesthesia and Analgesia in Laboratory Animals	6/26/16	
Animal Escape and Notification	6/26/16	
Blood Sample Withdrawal	6/26/16	
Breeding of Rodents	6/26/16	2/9/18
Counting the Number of Animals on a Protocol	6/26/16	
Food and Water Restriction	6/14/17	
Handling, Preparing, and Using MS-222	6/26/16	
Humane Endpoints	11/28/17	
Injections in Laboratory Animals	6/26/16	
Invertebrate Animals in Research	6/26/16	Discontinued 11/28/2017
Isolation of Sick Animals	12/16/16	
Recordkeeping and Perioperative Care and Monitoring	6/26/16	
Reporting Adverse Events	6/26/16	
Restraint in Animal Teaching and Research	6/26/16	
Survival Surgeries	6/14/17	
USDA Pain and Distress Categories in IACUC Protocols	6/26/16	
Use of Expired Products	12/16/16	
Veterinary Drug Labeling	6/26/16	
<b>ANIMAL DISPOSITION</b>		
Euthanasia of Laboratory Mice and Rats	6/26/16	
Protocol Closure or Transfer in the Event of a PI's Absence	6/26/16	
<b>ANIMAL FACILITIES AND HOUSING</b>		
Animal Husbandry, Care, and Documentation	11/28/17	
Animal Identification	6/26/16	
Approval of New Animal Facilities	6/26/16	
Cage Card System	1/18/18	
Group Housing of Social Species	6/26/16	
Holding of Animals not on Active Research Protocols	6/26/16	
Housing and Environmental Enrichment of Laboratory Animals	6/26/16	
Mice Cage Sizing and Density	6/26/16	
Non-Vivarium Animal Housing	6/26/16	
Quarantine and Stabilization of Research Animals	6/26/16	
Tracking Animal Use	11/28/17	
Transportation of Research Animals	6/26/16	
<b>INSTRUCTIONAL PROTOCOL SPECIFIC</b>		
Animal Use in Learning or Teaching	6/26/16	10/21/16

# LIBERTY UNIVERSITY

## INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

<b>MISCELLANEOUS</b>		
Inter-Institutional Animal Research	6/26/16	
Using Animal Material Obtained from Slaughterhouses & Processing Facilities	6/26/16	Discontinued 11/28/2017
<b>POST APPROVAL MONITORING</b>		
Congruence for NIH Funded Animal Studies	6/26/16	
Non-Compliance	6/26/16	
Reporting of Animal Concerns	6/26/16	10/21/16
Reporting to External Agencies	6/26/16	11/28/17
Review of Animal Welfare Concerns	6/26/16	
<b>PROTOCOLS</b>		
Administrative Review of Protocol Amendments	6/26/16	
Amendments Requiring a New Protocol	12/16/16	
Closure of Active IACUC Protocols by PI Request or Expiration	6/26/16	
Eligibility and Responsibilities of the Principal Investigator	6/26/16	
Exempt Animal Protocols	11/28/17	
Genetically Modified Animals	6/14/17	
IACUC Protocol Submission	6/26/16	
Performing a Literature Search for Alternatives	6/26/16	
Procurement of Laboratory Animals	6/26/16	
Use of DMR Subsequent to FCR for Protocol Modification Requests	10/21/16	
Use of Literature Searches for Alternatives	6/26/16	
<b>TRAINING AND PERSONNEL</b>		
Health and Safety	6/26/16	
Short Term Researcher Training	6/26/16	10/21/16
Training of Principal Investigators, Researchers, and Animal Care Staff	6/26/16	11/28/17