


TAMU Institutional Animal Care and Use Committee (IACUC) Protocol Review Checklist – AUP Help Text Addendum

The **Help Text** reproduced here may also be found in iRIS where the  symbol is displayed. **Help Text** provides additional information or instructions to the user to ensure that the response provided for IACUC review is complete and addresses all regulatory requirements. Example or recommended text that may be used to format a response in the corresponding field or section may also be available.

IACUC Guidance referenced throughout the AUP may be found here (requires TAMU authentication):
<https://vpr.tamu.edu/animals-in-research-and-teaching/texas-am-iacuc-guidance/>

The following **AUP Tool** may be helpful when drafting select portions of the AUP (requires TAMU authentication):
<https://vpr.tamu.edu/animals-in-research-and-teaching/texas-am-iacuc-guidance/>

- AWO-F-032 AUP Tool – *Offline AUP Worksheet*

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FUNDING

Examples of internal funding:

Departmental, start-up funds, T3 grant, X grant

Reasons for grant not fully congruent with AUP:

1. Several funding sources are utilized for this AUP. The funding source will be included for each experiment outlined in the Experimental Design section.

OR

2. Some procedures/ animal models/ animal numbers outlined in this AUP are not congruent with that of the grant proposal, but are related to these projects and involve similar procedures and outcome measures which are needed to collect data for future studies. Funds from internal sources will be used to pay for work not outlined in the proposal. Experiments covered by the grant(s) will be indicated in the Experimental Design section. All other experiments listed in that section will be funded internally.

PERSONNEL

Examples only. Copy desired text, paste to appropriate column in the table in section 6.1, and customize for each staff member.

Animal activities (duties) – column 2	Experience, Training, Education (Qualifications and plans for training if no experience) – column 3
As the PI, Dr. XXX is responsible for project design and performing all procedures outlined in the study including surgery, ICV injections, health monitoring, and euthanasia, as well as training & supervising staff.	Dr. XX has a PhD in Genetics and 20 years of experience handling mice and performing procedures listed in this AUP; including basic injections, surgery, and euthanasia; 3 years of experience performing ICV injections. Completed Working with the IACUC in CITI.
Perform all experiments outlined in the study including surgery, ICV injections, health & weight monitoring, and euthanasia.	Ms. xxx has a B.S. in Cell Biology. She has 1 year of general experience working with mice including basic husbandry, basic types of injections, and euthanasia. The PI will provide training and confirm proficiency with ICV injections. Completed applicable species/activity-dependent CITI & CMP courses.
Perform basic injections and monitor weight only.	Ms. X is an ungraduated student. She has no experience working with mice or performing the procedures listed in this AUP. She has completed applicable species/activity-dependent CITI & CMP courses. The PI or experienced lab staff will provide lab specific training and confirm proficiency.
CMP professional personnel will provide maintenance and management of the mouse colony and perform daily health checks, genotyping, and euthanasia procedures. Other tips: •Recommend listing the colony/herd manager as personnel <i>individually</i> for access to the AUP. •For Ag Teaching AUPs utilizing chute: Who will work the chute? Address training/experience.	(It is not necessary to provide qualifications of personnel performing professional activities as a function of a service unit (staff from ANSC, CMP, CVM, TIPs, VMP))
Ms. X is a study contact only and will not perform animal activities.	Experience and education not required if no animal contact.

Notes:

See TAMU-G-029 Guidelines for Animal Protocol Participation and Handling for new training requirements for non-PI lab staff (species & activity dependent). Effective for AUPs approved after Jan 1, 2021.

- CITI courses:
 - Working with the IACUC
 - Species course for *each* species listed on AUP
 - For rodents: Post-Procedural Care of Mice and Rats in Research: Minimizing Pain and Distress
 - For surgery: Aseptic Surgery (may be replaced by CMP/ARU/PAR/PRF live training)
- For work with rodents: TrainTraq course 2113938 Animal Allergens - BOHP
- For work with pregnant sheep: TrainTraq course 2111497 Researchers Who Work with Pregnant Sheep Inside Facilities - Biosafety.

LAY SUMMARY

AGRICULTURAL STUDY EXAMPLE:

Commercial feed additives commonly used in the production of poultry and other livestock include supplemental nutrients, digestive enzymes, anti-parasitic/anti-bacterial drugs, and functional feed additives (e.g., Probiotics/Direct-



Fed Microorganisms, Prebiotics, and Botanical Extracts). Livestock animal feeds are routinely formulated to contain these feed additives in order to improve animal health, feed efficiency, and growth performance which results in reductions to the overall cost and environmental impact of livestock animal production.

Our research will evaluate the efficacy of various combinations of feed additives with the benefit of developing poultry feeding and management programs able to improve animal health and reduce the overall cost of food animal production in order to provide a wholesome, healthy, inexpensive, and safe protein source for the consumer. The products we will evaluate will either be Generally Recognized as Safe or approved for use in animals by the Food and Drug Administration. Additionally, we will also conduct studies investigating the molecular/genetic basis for the functionality of these feed additives in order to support the further development of more effective feed additives.

BIOMEDICAL AUP EXAMPLE:

Repair of the injured adult nervous system is an immensely challenging goal. Rather than one 'magic bullet' cure, effective treatment of spinal cord injury (SCI) will likely require a multi-faceted approach combining multiple therapeutic strategies. Our laboratory is interested in exploring the potential of not only stem cell transplantation, but also gene therapy approaches to promote greater regrowth of damaged nerves in the spinal cord. These proposed experiments are designed to gain a better biological understanding of each approach. First, we seek to develop a greater understanding of how stem cells can 'replace' damaged spinal cord tissue. Second, we will test the ability of growth-promoting genes to enhance regrowth of injured spinal cord nerves.

CLINICAL TRIAL EXAMPLE:

Lymphoma is one of the most common cancers of the pet dog representing 83% of cancers that are derived from blood cells and 24% of all cancers seen in the typical veterinary oncology (cancer) clinic. This disease has a rapid and aggressive clinical course with a median survival time of 4-6 weeks without treatment and only 10-12 months with aggressive chemotherapy. New therapies are desperately needed to improve survival of this important disease.

RTI is a novel agent initially used as an anti-tuberculosis therapy in humans. This active compound was found to improve response to therapy in cell lines that were previously determined to be resistant to both radiation and chemotherapy. As drug resistance is one of the most important problems facing canines with lymphoma, this drug is an attractive addition to standard chemotherapy protocols. Additionally, this class of drugs have been used in high doses chronically in humans with no long term side effects and safety studies have been performed in canines using this specific formulation of the drug (see attached references).

The purpose of this study is to determine if a novel agent, RTI, is capable of improving the duration of the first remission to chemotherapy in canine patients with lymphoma. Client owned dogs meeting the enrollment/eligibility criteria would be recruited from the cases presented to the Texas A&M Veterinary Oncology.

TEACHING AUP EXAMPLE:

[Enter Course # and Title]

The objective of the laboratory component of this course is to instill an understanding of the skills required for breeding soundness evaluation of the male, artificial insemination, and pregnancy determination of common agricultural species. The laboratories include a combination of student participation to experience the skills required for reproductive management techniques with cattle and observation of the required skills demonstrated to the students with sheep and horses. Enrollment ranges from 12 to 20 students per lab section with 7 sections during the Fall and Spring and 2 sections during the Summer each year.

Breeding Soundness Evaluation of breeding sires. Students will be instructed in the techniques required to assess breeding soundness of males of various livestock species and to evaluate semen traits including motility, morphology, and concentration of spermatozoa. Semen will be collected from a bull, a stallion, and a ram.

Artificial Insemination. Students will be instructed in the recto-cervical technique for artificial insemination of cattle. Students will observe the cervical technique for insemination of the ewe and vagino-cervical technique for insemination of the mare.



Pregnancy Determination. Students will be instructed in the proper technique and skill required for determination of pregnancy in cattle via palpation per rectum. Students will observe the procedures for determination of pregnancy via diagnostic ultrasonography in a cow, ewe, and mare.

WILDLIFE AUP EXAMPLE

The purpose of this research is to understand the habitats used by Alligator Gar as a means for improving management and conservation of the species. By tagging fish and following their movements, we will gain information on how far fish move and with which habitats they associate. This information will be used by the state regulatory agency, Texas Parks and Wildlife Department, to assess how local management might be used to protect fish from harvest as well as how river flows should be managed to ensure appropriate habitats are available.

ANIMAL JUSTIFICATION

EXAMPLES

1. Anatomical and physiological complexity: The overall goal of this research is to investigate mechanisms of spinal cord injury, and so this research topic inherently requires the use of vertebrate species. Furthermore, non-mammalian vertebrate animals (such as frogs or zebrafish) are insufficient for addressing the specific questions of this research, because these species can regenerate their nervous systems after injury. Like humans, mammalian spinal cords do not regenerate following injury, and therefore rodents are the most appropriate models to study the pathophysiology of human injury.

2. Lack of computational models: Spinal cord injury and repair in mammals is an extremely complex phenomenon, and little is presently understood regarding the underlying mechanisms that can support repair of the spinal cord in the adult animal. Due to this remarkable complexity, which is only partially understood and quantified, no computational model is capable of modeling such a system; i.e., if the system is not understood, it cannot be made into a computer program.

SPECIES JUSTIFICATION

EXAMPLES:

- The species is the lowest that has transgenic animals and/or reagents required for the study/model
- The species is the lowest that has equivalent physiology and or structural features for comparisons to humans
- The species is the lowest for an established animal model that is required for comparative research
- The species is the lowest to adequately represent humans during surgery
- The species is the lowest in which a surgically implanted device can be used
- The species is the target animal (agriculture, wildlife) and a lower species is inappropriate for the study
- Other [Describe]

ANIMAL NUMBER JUSTIFICATION

Provide justification for the use of separate control groups within an experiment and replicates.

Select the type of rationale that best fits your individual experiments:

1) For group size determined by use of a statistical method/formula/program: Indicate the aim/study and the group size. Include the following information:

- Specify a hypothesis test.
- Specify the significance level of the test. Usually the alpha = 0.05 for the Type 1 error.
- Specify the smallest effect size that is of scientific interest.
- Specify the standard deviation or estimate the expected standard deviation for the control group.
- Specify the intended power of the test. The power of a test is the probability of finding significance if the alternative power is true. A power of 0.8 or greater is desirable.

2) For group size determined by reference to a similar study, where a statistically meaningful result was obtained: Indicate the aim/study and the group size. Cite the reference.

3) For group size determined by reference to your similar unpublished study/previous work: Indicate the aim/study and the group size. Provide a brief overview of the study and the data and whether statistical significance was achieved. Do not state 'in my experience', or similar, as the sole justification. Use a published study where possible.

4) For pilot study using small numbers of animals to study feasibility or proof of concept: Indicate the aim/study and the group size. Cite the reference. See TAMU-G-030 Guidelines for Pilot Studies.

5) For tissue harvest for in vitro studies: Indicate the aim/study and the group size. Animal numbers should be determined as follows:

- Determine the amount of tissue/cells required for the aim/study (e.g., 5×10^6 cells are required for each test; 60 tests are required; 3×10^8 cells are required for the aim/study).
- Determine the number of animals required for that amount of tissue/cells (e.g., each mouse provides 1×10^8 cells; 3 mice are required)
- Justification must be provided for the amount of tissue required. Please note that animal numbers cannot be justified on the basis of how many experiments the lab personnel can perform in a day, week, month, etc.

6) For teaching protocols: Indicate the aim/study and the group size. Animal numbers should be justified by a specified student-to-animal ratio (e.g., each animal will be handled by a maximum of 4 students. There are less than 20 students every semester and the class will be taught 3 times during the protocol approval [$n=20/4 \times 3=15$])

7) For wildlife protocols (do not include observation only): Indicate the aim/study and the group size. Animal numbers should be the maximal number of animals expected to be captured, handled or otherwise used over the duration of the protocol. The minimal number of animals required for statistical significance must still be justified, unless this is a survey type project. If necessary, reference to historical data can be used to justify the number of animals.

8) For all other justifications: Indicate the aim/study and the group size. Describe why a scientific justification is not possible and how the number of animals needed was determined.

EXPERIMENTAL DESIGN

Please note: The following AUP Tools may be helpful when completing this section (requires TAMU authentication):

<https://vpr.tamu.edu/animals-in-research-and-teaching/texas-am-iacuc-guidance/>

- AWO-O-036 AUP Tool - Procedure Flowchart/Timeline
- AWO-O-069 AUP Tool - Experimental Design and Timeline

Experiment 1: Provide a short 1-2 sentence description of why you are performing this experiment. **Do not include Procedure descriptions/methods in this section.** List funding source when more than one source is listed on the protocol; e.g.: R01 NS123456; American Heart Association.

List the experimental timeline. Write this from the animal's point of view - what is happening to the animal and when? Use a **bullet point** for each step, e.g.:

- *(not in iRIS) Acquisition, quarantine or acclimate to facility (minimum X days)*
- [specify animals to be used] will undergo [first step/procedure] - Begin the timeline with breeding/animal ID/genotyping (if applicable)
- ~[X hours/days/weeks/months; when something occurs] after [first step], animals will undergo [second step/procedure]
- ~[X] after [previous step], animals will be administered [e.g. 1 of 3 doses < X mg/kg name of test compound, route, maximum volume in ml/kg]
- ~[X] after [previous step], animals will be administered [e.g. 1 of 3 test compounds]
 - Compound 1 (dosage, route and maximum total volume of injection in ml/kg will be described in the Drug/Agent section)
 - Compound 2 [same as above]
 - Compound 3 [same as above]

- ~[X] after [previous step], animals will undergo [e.g. 1 of 3 behavioral assessments or any 3 behavioral tests listed in Materials and Methods. List frequency, duration, and interval if not listed in Procedures: e.g. < X tests/week for X weeks, with at least X hours/days between tests]
 - Behavioral assessment 1
 - Behavioral assessment 2
 - Behavioral assessment 3
- ~[X] after [previous step], animals will undergo [surgery] (see [list surgery name from Surgery page] surgery) to[do what?].
- [Specify endpoint relative to previous step; e.g.: time point or when tumors reach a specific size] animals will be euthanized and tissues harvested for [briefly list the tests that will be performed (e.g.: Prostate tissue will be harvested to perform PCR)]. [Indicate when animals that do not meet the study endpoint parameters are euthanized.]

Animal numbers:

- Provide a formula that addresses all parameters described in the experimental time line; e.g.: 6 mice/group x 2 sexes x 3 compounds x 3 doses x 2 surgeries x 1.33 (25% surgical failure) x 3 repetitions = **862 mice** [always round up!]
- Two or more formulas for the same experiment need to be listed as separate bullet points.
- Do not provide justification for the group size or the animal numbers here. This is in a previous section.

Experiment 2: Example of a standalone aim used for training. This aim will be used for training new or existing personnel on new procedures in the lab. Naive animals or animals that have undergone procedures with only minimal pain and distress [define what this looks like on your protocol] may be used for training purposes to allow lab personnel to optimize surgical, injection or other procedures. Include number of animals for training.

- Equal volume saline will replace test compound administration for injections where possible
- For practicing surgical procedures, if two or more practice surgical procedures are applied to an individual animal, the practice surgeries would be performed as a non-survival surgery and the animal would be euthanized without regaining consciousness. Non-survival surgeries may be performed without aseptic technique.
- Some surgeries require the animals to be allowed to recover from anesthesia in order to identify potential issues with the practice, e.g.: [list]. In this case, the surgical procedures will be performed aseptically and approved post-operative care, including analgesia, will be administered. Animals will be clearly labeled as practice animals.
- Animals will be euthanized within [X] days after the practice procedure.

Animal numbers:

- Provide an animal number formula for the projected number of animals used for training; e.g.: 2 rats/group x 2 surgeries x 3 trainees = **12 rats**; OR
- State 'No additional animals are requested.'

Repeat process above until all experiments have been described. Feel free to combine experiments where the activities are the same.

Exp. Design Example

Experiment 1-1: Examination of the osteogenic and myogenic responses of the Fine Branch Arboreal (FBA) experimental enclosure in the growing mouse. This exercise regimen results in low-impact, multi-directional mechanical loading on the musculoskeletal system, which may be an important treatment strategy for improving bone and muscle health in individuals with elevated fracture risk.

Funding: Grant from XYZ, Maestro# Mxxxxxxx.

Sequential list of procedures:

- Acquisition from vendor
- Acclimation at CMP for minimum of 3 days
- Random assignment to FBA or control environment groups (water flood level in floor of FBA environment < 2 cm)
- Anesthesia for tail vein blood draw and kinematic marker placement, recovery, locomotor kinematics analysis, ear punching/notching for identification
 - At baseline and monthly for up to ~4 months

- Recovery period of at least 1 day
- Anesthesia and in vivo musculoskeletal imaging (pQCT) followed immediately by bone densitometry scanning (DEXA)
 - At baseline and monthly for up to ~4 months
- Recovery period of at least 1 day
- Anesthesia and in vivo muscle function analysis (ankle torque) of one leg followed by SC injection of bupivacaine and lidocaine (2 separate injections) prior to in vivo bone material property analysis (BioDent) of the other leg
 - At baseline and monthly for up to 5 months
- At the end of Week 1, water flood level in floor of FBA environment raised to ~10 cm
- 1st IP injection of calcein for dynamic histomorphometric analysis ex vivo ~12 days prior to sacrifice
- 2nd IP injection of calcein for dynamic histomorphometric analysis ex vivo ~4 days prior to sacrifice
- Approximately 24-48 hrs after the final in vivo testing timepoint, mice will be anesthetized for a non-survival surgery to isolate muscles for in vitro testing followed immediately by euthanasia.

Total animals for Experiment 1-1 = 40 C57BL/6 mice (2 groups x 20 mice/group). Two experiments of 10 mice/group will be conducted in series in order to reduce error associated with building multiple FBA experimental enclosures.

Notes:

For renewals, incorporate information from standalone amendments/VVC approved on the previous study. To access those amendments, see IRIS-M-017 How to View Old (Standalone) Amendments [PI]

PROCEDURES

Details and descriptions of procedures go here. Just the names of the procedures go in the Procedures Table.

Other notes:

- Only a broad overview of surgical procedures should be included here. Surgery details will be captured in the Surgical section
- For Privately Owned Animals, describe only those procedures that differ from Standard of Care
- Include husbandry practices, vaccinations, parasite monitoring, de-worming, etc. if not provided by CMP or other core unit. Do you have a facility husbandry SOP to copy here? Does CVM/TIPS/ASTREC/VMP/ARU/PAR/PRF provide these services?
- What enrichment is provided? If enrichment is provided by CMP/CVM/TIPS/ASTREC/VMP/ARU/PAR/PRF, just make that statement. See TAMU-G-027 IACUC Guidelines on Enrichment, Single Housing - Biomedical Species.
- Give yourself flexibility – consider providing a range for blood collections, drug doses, etc. and don't specify needle gauge.
- For wild animal capture explain how you will handle unintended/by-catch.
- For renewals, add procedures from previous AUP amendments/VVC. To access those amendments, see IRIS-M-017 How to View Old (Standalone) Amendments [PI].

If Breeding is on AUP, address the following:

Breeding scheme (pair, harem, trio)

Describe synchronization protocol for artificial insemination or embryo flush/transfer.

Assessment of pregnancy/reproductive stage? Vaginal smear or visual inspection of vaginal/sperm plug, ultrasound for larger animals (describe procedure)

Animal Identification: Permanent marking (tattoo), microchip implantation, ear punch, ear tag or other invasive method should be described. Can provide flexibility by listing all methods that may be used. See TAMU-G-014 IACUC Guidelines for the Identification of Research Animals.

Genotyping: Tail snip, up to 2mm before age 21 days, as described in TAMU-G-007 Guidelines on Genetically Modified Animals and Genotyping. Describe what happens to animals that express a negative or unwanted genotype?

Blood Collection:

List method(s) here (maximum volume collected per draw & frequency goes in the Samples collected section below). Provide flexibility by using statements such as 'blood collection will occur no more often than X'. Blood volume may also be expressed as % Circulating Blood Volume (CBV). See TAMU-G-017 IACUC Guidelines on Blood Collection for more information regarding acceptable quantity and frequency of blood sampling in rodents and other species as well as guidelines for specific methods of blood collection. Indicate if animals are anesthetized for blood collection and list drug name. Relevant anesthesia information should be described in the **Agents** section.

Behavioral or Nociceptive Testing:

List name and description of device; e.g.: size, materials used for construction, safety precautions, etc. Describe drying and warming process for water mazes. Describe acclimation and testing procedures. List duration of procedures. Are animals 'housed' in the device? List frequency of procedures if not described under the individual time line. Describe removal criteria or criteria for halting testing for painful or distressful tests; e.g.: shock or heat testing, water mazes.

Euthanasia: See TAMU-G-025 and TAMU-G-028 for more information.

Describe euthanasia procedure. Include method of ensuring death. Provide justification for varying from the approved methods as described in the current AVMA Guidelines for the Euthanasia of Animals. An Adjunctive Method is a species-specific mode of assuring death that may be used after an animal has been made unconscious. Include all anesthetics, sedatives and euthanasia compounds used. Physical Methods of euthanasia include: exsanguination, decapitation, cervical dislocation, bilateral thoracotomy, removal of vital organ(s), pithing, penetrating captive bolt and others as appropriate per species. Note: A perfusion of short anesthetic duration without concurrent procedures is considered euthanasia (not a non-survival surgery).

Hazards (other than radioactive materials): Same as Test Compounds. Make sure to include the hazard in the Drugs/Agents table and list it in the Chemical Safety Details section.

Hypoxia Chamber/Other Environmental Manipulation Chamber:

List & describe method of environmental manipulation. Describe caging used to house animals. Is this the standard CMP caging for the species? Describe how husbandry will be performed while animals are in the unit. Indicate who will provide daily care. (Address frequency of monitoring in the Monitoring & Recording section and removal criteria in the Animal Removal section). Describe frequency and duration animals will be in the chamber here or under the individual experimental timeline. Provide flexibility by using statements like 'Animals will be in the X chamber for up to X weeks no more than every other month'. Attach a copy of the maintenance record that will be used for this piece of equipment. This should address all stages; e.g.: prior to starting experiment, while experiment is underway, quarterly/biannually/yearly activities.

Imaging:

List imaging method/modality. Indicate if animals are anesthetized and list drug name. All other relevant anesthesia information should be described in the Non-surgical Anesthesia section. Contrast (if used): Include route/dose, etc. in the Drugs/Agents table. Indicate if hair removal is performed. If so, state method(s) of depilation. If depilatory cream is applied, include statement of its removal and include in Drugs/Agents table.



Describe frequency here or under the individual experimental time line. Provide flexibility by using statements like 'imaging will occur no more often than X'.

Irradiation (non-imaging):

Indicate type and maximum dosage of radiation.

Indicate if animals are anesthetized and list drug name. All other relevant anesthesia information should be described in the Drugs/Agents table.

Indicate if hair removal is performed. If so, state method(s) of depilation. If depilatory cream is applied, include statement of its removal and include in Drugs/Agents table.

Describe frequency here or under the individual experimental time line. Provide flexibility by using statements like 'irradiation will occur no more often than X'.

Make sure to list type of radiation used in the Radioactive Materials section.

Non-surgical Fasting or Fluid Restriction: Indicate if animals undergo fasting or fluid restriction. Description and justification should be provided in the Husbandry section (select Special Husbandry).

Non-surgical Anesthesia:

Include anesthetic drug. List drug, dosage and route in the Drugs/Agents table.

Indicate that ophthalmic ointment is used or provide justification for lack of use. List ointment on the Veterinary Drugs page.

Indicate that thermoregulatory support** is used during the procedure and recovery. Describe mechanism used for support. Provide justification for lack of thermoregulatory support.

Indicate if fluids are provided and list compound. List compound, dosage, route and frequency in the Drugs/Agents table.

Describe recovery procedures; e.g.: Animals are recovered in a clean cage without bedding, placed half-on/ half-off a [SPECIFY HEAT SOURCE] with the unconscious animal on the warm side, and returned to the home cage when fully ambulatory.

Indicate the total time under anesthesia.

Routes of Administration:

List doses in mg/kg for test compounds. This information will be captured in the Drugs/Agents table.

List route and maximum volume administered in ml or ml/kg

Indicate if animals are anesthetized and list drug name. All other relevant anesthesia information should be described in the Drugs/Agents section.

Special Diet:

List name or type of diet, manufacturer, and duration of diet in the Husbandry section (select Special Husbandry)

Special Water:

List compound to be administered in drinking water. Include dose and frequency for test compounds. Include the drug, dose and frequency in the Drugs/Agents table.

Test Compounds:

List the name, maximum dosage in mg/kg, route and maximum total volume of injection in ml or ml/kg (if not described under Routes of Administration)

List non-USP compounds in the Drugs/Agents section (Non-Pharmaceutical Grade fields).

Wildlife Capture

Provide a description of trapping and restraint equipment used (bare hands, bucket, mist net, sherman live trap, etc.). Describe methods used to minimize handling stress when animals are removed from the capture mechanism and manipulated. Include duration of time animal will spend in the restraint or trapping mechanism. Indicate if animals are able to make normal postural movements while in the trap.

For non-manual trapping please include the following in the description:



Where will traps be placed in the environment?

How long will traps be in place? One hour; 12 hours?

How often and at what time will traps be checked? Address how the animal will be appropriately sheltered/protected from inclement weather and predators.

How will researchers minimize the potential harm caused to the animals by the trap?

Describe food/water source to be provided in the trap if animal is to be left for an extended period of time. Provide justification for lack of source.

Are traps designed to prevent capture of non-targeted animals? If not, how will non-targeted animals be handled? If applicable, description of how the animals will be transported to/from capture site to research location should be included in the Field Studies section.

**State how often any procedure will be performed behavioral testing, imaging, irradiation, etc. For flexibility state "performed no more often than X"

For Procedures Table

- Procedure names can be listed in one line per location.
- Include husbandry practices when not provided by a core unit.
- Indicate "off-campus/ field location" in rooms column for field location
- Use "AA" for vivarium or core unit location only. Must list a room number for PI lab or non-centralized/satellite location.

PROLONGED RESTRAINT

Prolonged restraint is an exception to the Guide/ care standards and justification of use is required. List duration and frequency of restraint, acclimation to restraint to method or device, and removal criteria for those that do not adapt.

Describe prolonged restraint only. Do not include restraint for routine clinical or experimental procedures. Please refer to pages 29-30 of the Guide for the Care and Use of Laboratory Animals (<https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf>) for further details.

The following are important guidelines for restraint:

- Restraint devices should not be considered a normal method of housing, and must be justified in the animal use protocol.
- Restraint devices should not be used simply as a convenience in handling or managing animals.
- Alternatives to physical restraint should be considered.
- The period of restraint should be the minimum required to accomplish the research objectives.
- Animals to be placed in restraint devices should be given training (with positive reinforcement) to adapt to the equipment and personnel.
- Animals that fail to adapt should be removed from the study.
- Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.
- Veterinary care must be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates the temporary or permanent removal of the animal from restraint.
- The purpose of the restraint and its duration should be clearly explained to personnel involved with the study.

Note: For Wildlife trapping/capture, if animals are unable to make normal postural movements if in the trap for an extended period of time, this may constitute prolonged restraint.

For each animal or study group requiring this exception, identify the specific group and indicate the number of animals involved. Include any plan to return animals to standard environmental conditions.

Clinical Signs (Anticipated Effects)

Notes/tips: The more robust this section, the less likely you'll need to report an unanticipated adverse event.



- Side effects
 - For cattle: Include side effects of chute injury.
 - If CRRC form exists, this section should match discomforts and risks in the consent form.
- Monitoring
 - If weight loss listed above, how often will animals be weighed? Daily? If tumors measured, how often?
 - Will CMP/VMP/ARU/PAR/PRF staff perform some of the monitoring?
 - Might BCS only be recorded if below/above a certain score?
- Removal
 - “Animals with signs of [XYZ] will be seen by the veterinarian or euthanized.”
 - For use of teaching colony or herd, recommend a statement that the colony/herd manager will monitor health records and assign animals to teaching activities to prevent over-use. Colony/herd manager should be listed as personnel in 6.1 for access to the AUP.
 - For cattle/Ag: Add removal for animals that do not stand calmly in chute for duration of procedures.

Clinical Sign - Category	Clinical Sign - Detail	Early Removal Criteria/Criteria for Moribundity
Changes in general appearance	Abnormal Posture, Discoloration of Feces, Discoloration of Fur, Discoloration of Urine, Lack of Grooming, Nasal Discharge, Ocular Discharge, Prostration, Rough Coat, Swelling	Impaired mobility or lesions interfering with eating, drinking or ambulation. Icterus (jaundice; yellow color to skin). Markedly discolored urine, polyuria, or anuria if prolonged (>3days). Impaired mobility or lesions interfering with eating, drinking or ambulation. Paralysis. Persistent lateral recumbency. BCS* of <2. ERS** of > or = 3.
Changes in appetite or weight	Decreased Appetite, Increased Appetite, Weight Loss: <10%, <20% (provide scientific justification)	Extended period of weight loss progressing to emaciation with BCS* of <2. Rapid weight loss of >20% in 1 week. ERS** of > or = 3.
Changes in respiration	Labored, Rapid, Slow, Shallow, Sneezing, Wheezing	Persistent cough, rales, wheezing, nasal discharge. Respiratory distress (dyspnea) or cyanosis
Changes in behavior/ neurologic status/ musculo- skeletal	Agitation, Depression, Hyperactivity, Hypoactivity, Lethargy, Spasticity, Tremors, Impaired Ambulation, Muscle Atrophy, Paralysis [Limb or other (describe)]	Central nervous system signs (head tilt, tremors, spasticity, seizures, circling or paresis) with anorexia and hindering of animal's ability to obtain food/water. Impaired mobility or lesions interfering with eating, drinking or ambulation.
Tumors, skin conditions	List % of total body weight or size. Justify tumor burdens exceeding 10% Total Body Weight. Tumor necrosis (<1cm mice, <2 cm rats) with ulceration and dry scab within 24 hours	Tumors that are >10% and <20% of animals original body weight (provide justification for tumor burdens that will exceed 10% of total body weight). Tumors >2cm in mice or >4cm in rats. Ulcerated tumors that are moist and not healing (>1cm mice, >2cm rats). Extensive necrotic tissue or skin ulceration with >10% body surface affected. Persistent self-induced trauma. Tumors which significantly impede ambulation and/or cause significant pain or distress. Tumors which hinder ability to obtain food/water.
Other clinical signs	Bleeding, Blindness, Coma, Constipation, Diarrhea, Hyperthermia, Hypothermia, Infection, Self-Induced Trauma, Other [provide description]	Icterus (jaundice; yellow color to skin). Markedly discolored urine, polyuria, or anuria if prolonged (>3days). Persistent self-induced trauma. BCS* of <2. ERS** of > or = 3.
Injury or Mortality for Captured Wildlife	Address historical animal injury and mortality rates associated with your capture and animal handling methods and methods taken to eliminate such injuries, etc.	Describe your plan for providing veterinary care or humane euthanasia for animals that experience a serious injury or illness.
	*Body Condition Score (BCS)	**Early Removal or Moribundity Score (ERS)
	1 - Emaciated 2 - Under-conditioned 3 - Well-conditioned 4 - Over-conditioned 5 - Obese	1 - Normal appearance 2 - Decreased mobility, weight loss, lack of grooming 3 - Extreme lethargy, body weight loss of >=20%, unresolved dehydration >=10% 4 - Non- responsive to stimulation

Please note: The following AUP Tool may be helpful when completing the *Animal Removal from Study* section (requires TAMU authentication): <https://vpr.tamu.edu/animals-in-research-and-teaching/texas-am-iacuc-guidance/>

- AWO-O-072 AUP Tool - ERC Worksheet



DRUG/AGENT ADMINISTRATION

Drug Table

- For drafts copied from previous AUP, re-enter agents (if missing).
- For renewals, check for agents added to previous AUP via amendment/VVC. To access those amendments, see IRIS-M-017 How to View Old (Standalone) Amendments [PI].
- Instead of “as needed”, define use. For example, “penicillin given once prophylactically during castration”.

NPGS assurance

Example: For all compounds, a neutral pH will be achieved by dissolving the solute in PBS (pH 7.4). The sterility of compounds will be controlled by working in a biosafety cabinet and filtering the dissolved compounds through 0.2 micron filter. The stability of each compound will be controlled by storing at refrigeration temp., using working dilutions only once, within a week of dilution from the stock.

NPGS justification:

Sample exception reasons for non-pharmaceutical grade compounds: Copy and paste all that apply. List compound(s) that relate to each type of justification selected.

- Use of a non-pharmaceutical grade anesthetic is requested (Avertin, Sodium Pentobarbital, Inactin, Urethane) [provide justification for use]
- Compounds are not available in pharmaceutical grade
- The pharmaceutical grades of the compounds are not in the correct formulation for the necessary route of administration
- The pharmaceutical grades of the compounds are formulated with additives that could confound the data obtained from the study
- Other [provide justification for use]

CAT D RELIEF MEASURES

Examples:

- Strict aseptic technique during the procedure, use of post-op supplemental warmth, extra bedding, monitoring the animal until mobile followed by daily observation.
- Positive interaction with care staff, crinkle paper & huts for mice; structures for goats to climb; substrate for fish, etc.

Consider providing **additional enrichment**, especially for singly housed animals. See TAMU-G-027 IACUC Guidelines on Enrichment, Single Housing - Biomedical Species.

Positive interaction with care staff, crinkle paper & huts for mice; structures for goats to climb; substrate for fish, etc.

CATEGORY E RELIEF MEASURES

A. Example: Injury to the spinal cord, as well as the laminectomy surgery itself, have the potential to result in unrelieved pain. Though inflammatory pain associated with surgery is likely to resolve over time, neuropathic pain resulting from spinal cord injury does not resolve in human patients or in animals.

B. Not giving analgesia for pain/distress is an exception to standard care, must give justification.

Example: The anti-inflammatory effects of analgesia would affect the outcome....explain.

C. Consider providing **additional enrichment**, especially for singly housed animals. See TAMU-G-027 IACUC Guidelines on Enrichment, Single Housing - Biomedical Species.

Examples: Positive interaction with care staff, crinkle paper & huts for mice; structures for goats to climb; substrate for fish, etc.

Other examples: “We will acclimate animals to the testing environments and to being handled by your staff prior to initiating the study. After a surgery, we will closely monitor animals’ health and activity levels twice daily and we will provide softened food on the floor of the cage and extra bedding during a surgery recovery period.”

EUTHANASIA

Help Text for Euthanasia Section

TAMU-G-025 Guidelines for the Performance of Physical Methods of Euthanasia in Mice and Rats

TAMU-G-028 IACUC Guidelines on Euthanasia Using Carbon Dioxide

TAMU-G-047 Guidelines on Inhaled and Noninhaled Agents of Euthanasia

Secondary Physical Euthanasia Methods for Lab Rodents and their Wild Counterparts (Wild Small Mammals) include: Cervical dislocation, decapitation, exsanguination, bilateral thoracotomy, removal of vital organ(s).

Immersion of fish in solutions of MS 222 for 30 minutes following loss of rhythmic opercular movement is sufficient for euthanasia of most fish. Alternatively, anesthetize fish and employ secondary method.

Adjunctive Euthanasia Methods for Rabbits include: potassium chloride, exsanguination, bilateral thoracotomy.

Adjunctive Euthanasia Methods for Fish include: decapitation, pithing, freezing, other physical methods.

Captive Bolt/Gunshot

- Include application of adequate restraint, monitoring for proficiency (ensuring effective application of the captive bolt/gunshot as indicated by immediate loss of consciousness lasting until death), and maintenance of bolt after each use.
- Signs of effective captive bolt penetration and death are immediate collapse and a several-second period of tetanic spasm, followed by slow hind limb movements of increasing frequency. The corneal reflex must be absent and the eyes must open into a wide blank stare and not be rotated.
- Eye and hearing protection is also recommended.
- Provide description of anatomical landmark of bolt/gunshot

FYI: Personnel must comply with laws and regulations governing the possession and discharge of firearms; local ordinances may prohibit the discharge of firearms in certain areas.

See the AVMA Guidelines on Euthanasia 2020 Edition: <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>

Notes:

- a) Perfusion procedures of short anesthetic duration without concurrent procedures are considered euthanasia.
- b) The performance of certain methods of euthanasia are conditional upon the demonstration of proficiency.
- c) Euthanasia methods should be fully described in the Procedures section and euthanasia drugs listed in the Drug/Agent Administration section.

SURGERY (SURVIVAL)

Description

Example:

1. We will weigh the mice, and start isoflurane administration via nose cone. For pain management, the mouse will be injected with Butorphanol during anesthesia induction.
2. We will shave the fur on the skull, place the mouse on the apparatus, and then apply sterile eye ointment. (nose clamp: should be used with very low pressure on the animal’s nose; to prevent breathing problem)



- Using a sterile scalpel, we will make a longitudinal incision from anterior to posterior through the scalp. Then clean the bregma, and lambda area and adjust head position equally. The skull will be kept moist with sterile saline.
- The injection syringe and needle will be placed into the holder of the stereotaxic arm. A pen will be used to mark the position of the injection.
- A small syringe needle will be used to carefully perforate the edges of the craniotomy.
- The syringe will be loaded with solution (up to 2 ml). Controls will be injected with up to 2 ml of saline only.
- We will bring the tip of needle to the lambda position and then we will calculate the X,Y positions.
- We will move the needle 2.5mm downward from the skull surface and inject the solution at a rate of 1ul per 30 seconds. The needle will be left in place for 5 minutes and then slowly withdrawn.
- The incision will be closed with vet bond. Additionally, the mouse will be allowed to recover on an insulated or water circulating heating pad (cage half on/half off heating pad so mouse can move away from the heat).

Monitoring Assessments:

Frequency

EX: Every 30 min for 4 hours post-op then twice daily for 4 days by research staff; then daily by CMP team, and weekly by research staff

Duration

EX: For 4 days after surgery, and then weekly throughout the study.

Monitoring Support (last column)

Frequency

EX: Analgesia given during anesthesia induction then every X hours if "Y" is observed. (Y = specific physical appearance, behavior, or clinical signs)

Duration:

EX: X hours post-op then as determined by observance of factors listed above

Potential Post-Op Complications

EX: The primary adverse event associated with bilateral femoral defect surgery is post-operative infection and includes redness, swelling, discharge, dehiscence (opening of the surgical site). Systemic signs of infection include lethargy, loss of appetite, weight-loss, or moribund status, and discomfort in most cases. Pain is also another potential adverse event. Signs of pain would include decreased activity, lethargy, and decreased appetite. Rabbits will be treated peri-operatively with extended release buprenorphine and an NSAID to manage post-op pain (see table in Drug/Agent section).

PHYSICAL SAFETY

High noise levels

EX: Some situations that may require hearing protection: Use of pigs, birds (aviary), or multiple dogs in kennel.

Sharps/physical hazard

EX: Sharps are used for blood collection. Needles will not be recapped and will be disposed in a puncture-proof sharps container.

Power Equipment

EX: Hydraulic chutes will be used to for cattle procedures. Proper training will be given/verified by the PI.

Electrical Hazard

EX: Fish housed in aquaria, ground-fault circuit interrupter GDFI outlets in use