# Animal Use Protocol Guidance

**What requires IACUC (Institutional Animal Care and Use Committee) approval**

IACUC review and approval is required for all research and teaching protocols involving the use of live vertebrate animals. This includes all protocols in which animals are manipulated or subjected to procedures that may cause pain and/or psychological distress, or which substantially alter the natural behavior(s) of the animals. This excludes animals that are dead when obtained by university personnel. It also excludes avian, reptilian, and amphibian embryos (prior to hatching), and purely observational studies in which the presence of the investigator(s) or investigative device(s) is not expected to significantly alter the behavior of animals in their natural environments.

Research and teaching activities involving agricultural animals, whether conducted by the University of Arkansas at Fayetteville (UAF) or the Division of Agriculture, will be reviewed by the [Division of Agriculture IACUC](https://aaes.uark.edu/technical-services/agiacuc/).

## Animal Use Protocol (AUP) Forms

**Biomedical and Sociobehavioral Research and Teaching:** This is research and teaching whose goal is to generate or communicate knowledge that will contribute to improving the health and well-being of humans and/or animals not involved in agricultural production. For biomedical or sociobehavioral research and teaching UAF endorses and will comply with the standards for animal care and use described in the current version of the Guide for the Care and Use of Laboratory Animals (“the Guide”), published by the National Research Council. Anyone conducting biomedical or sociobehavioral research and teaching activities with a live, vertebrate species not covered by these documents should work closely with the IACUC and the Attending Veterinarian (AV) to develop appropriate guidelines for the particular species being studied. Any study that involves procedures that induce pain or distress, involves surgery, or requires the use of medical intervention such as analgesics and/or anesthetics should use this form.

**Field Research and Teaching**: This is research and teaching whose goal is to generate or communicate knowledge that will contribute to our understanding of animals living in the wild or naturalistic enclosures, including zoos and animal sanctuaries. As defined in the Animal Welfare Act and Regulations, field studies are conducted on free-living wild animals in their natural habitat. It excludes any study involving invasive procedures or that harms or materially alters the behavior of the animal(s) being studied. The field form may be used for studies that will not take place on campus and do not involve surgical procedures as defined below or the use of analgesics or anesthetics to mitigate pain and distress. If the study involves capturing animals and moving them into a laboratory setting for manipulation, the Biomedical/Sociobehavioral form should be used.

**Instructions:**

* Submit your protocol in a timely manner

It is important to submit a protocol as far in advance of the IACUC meeting as possible.  This is especially important if you wish to have the IACUC Program Manager check the protocol for areas that need correction or additional information. The deadline for submitting an AUP is seven days prior to the date of the next IACUC meeting. Please see the [Animal Care and Use website](https://research.uark.edu/units/rscp/animal-care-and-use.php) for the date of the next meeting.

**Project Information**

* Accurately account for the number of animals needed for this AUP

The number of animals listed must agree with the total number of animals needed as described in Section II: Experimental Design. IACUC members do check this and will ask for corrections and/or clarifications if needed before approval is given. You also must provide a justification for the number of animals requested. For teaching protocols, the total number should account for how many times the class is taught during the protocol approval period. This information should also be clearly stated in the narrative under Experimental Design.

* If state or federal permits are required to perform the work, a copy of the permit(s) should be submitted with the AUP, or as soon as available.
1. **Abstract**
* Please keep the abstract to 300 words or fewer.

Include all required information in a format easily understood by a **non-scientist**.

1. **Experimental Design**
* Ensure that the number of animals, groups, and treatments are clearly described.

As noted in the instructions on the AUP Form, using tables listing groups with respective treatments and numbers of animals/group, etc. can be extremely useful in communicating your plan.

* Provide sufficient detail for review by the committee.

While many procedures are intuitively obvious to the investigator, they may not be so to all IACUC members. Be descriptive; assume that the reviewer is completely unfamiliar with your work. For example, in field research, banding birds implies a number of steps that may not be immediately obvious to a reviewer. Describe the steps involved in this procedure.

* Clearly state and describe terminal procedures.

Indicate when procedures are terminal; do not assume IACUC members will know.

1. **Pain and Distress Categories**
* Indicate the correct pain or distress level

Be sure that you read the description for each level. The level should match the procedures described in the “Procedures” sections of the AUP. The most common error is classifying procedures that produce an experimental infection; note that Level 2 includes infections that produce no significant pain or distress, and Level 3 includes infections that produce systemic disease resulting in pain, distress, or death.

* **Level 1** – Animals being bred, conditioned, or held for use in teaching or research but not yet used for such purposes, or observations of animal behavior without intervention.

Examples: Breeding colonies, nest observations.

* **Level 2** - Animals will only be subjected to procedures causing nothing more than minor discomfort (*e.g.,* injections, blood collections); neither pain nor distress will be induced, or animals will be humanely euthanized prior to any procedures that induce pain or distress. If analgesics are used, the project is at least Level 3.

Examples: Physical examination, physical methods of identification, behavioral training, live trapping with the potential for injury, short term restraint such as for transport.

* **Level 3** - Pain or distress will be relieved by appropriate therapy, *e.g*., sedatives, analgesics, anesthetics, or euthanasia.

Examples: Invasive and surgical procedures, induction of disease that causes pain or distress.

* **Level 4** - Drug intervention for pain or distress would interfere with the protocol. Specific justification MUST be provided.

Examples: Death as an endpoint without pain-relief, exposure to noxious chemicals or environment without ability to escape, prolonged restraint, prolonged food, or fluid depravation.

1. **Surgical Procedures (Guide, 115)**
* **Non-survival surgery** including tissue harvests, involves the euthanasia of the animal before recovery from anesthesia. At a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. For non-survival procedures of extended duration, attention to aseptic technique may be more important to ensure stability of the model and a successful outcome. (Guide, 118)
* **Survival surgery** is defined as any surgical procedure described below in which the animal is revived from anesthesia. Aseptic technique is mandatory for all survival surgeries. While explicit notation of major vs minor surgery is not required on the AUP form, the IACUC will use the distinction when evaluating the experience of the research team. Surgeries and procedures are only to be performed by individuals approved and trained to do so, and a proficiency evaluation can be requested by the IACUC or Attending Veterinarian at any time.
	+ **Major survival surgery** (*e.g*., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Guide, 117).
	+ **Minor survival surgery** does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time. (Guide, 117)

When attempting to categorize a particular surgical procedure, the following should be considered: the potential for pain and other postoperative complications; the nature of the procedure as well as the size and location of the incision(s); the duration of the procedure; and the species, health status, and age of the animal. (Guide, 117)

* **Multiple survival surgeries** on a single animal should be evaluated to determine their impact on the animal’s wellbeing. Multiple major surgical procedures on a single animal are acceptable only if they are (1) included in and essential components of a single research project or protocol, (2) scientifically justified by the investigator, or (3) necessary for clinical reasons. Conservation of scarce animal resources may justify the conduct of multiple major surgeries on a single animal, but the application of such a practice on a single animal used in separate protocols is discouraged and should be reviewed critically by the IACUC. (Guide, 30)
* Ensure that you have the appropriate license(s) for controlled substances used for anesthesia/analgesia.

Appropriate anesthetic must be used, and most anesthetics are controlled drugs. It requires both a state and federal license to purchase, keep, and use DEA controlled drugs. Also, if postoperative pain medication will be needed, more controlled drugs may be needed. These licenses take quite a bit of time to obtain, so plan appropriately. The process for obtaining licenses is described [here.](https://research.uark.edu/documents/rscp/dea_licensing.pdf) All investigators planning on using controlled substances must obtain the appropriate licenses and provide a copy to the IACUC office to be kept on file. Controlled substances can only be accessed and administered by those authorized and trained to do so in accordance with the license. Researchers must work with Environmental Health and Safety to ensure that all substances are stored and disposed of properly and that usage is well documented. For centralized animal facilities, consult with the facility manager or veterinarian for storage availability.

* Clearly describe the dosage of all drugs that are to be used.

See the details in the instructions on the AUP Form. NOTE: Expired medications may not be used.

1. **Non-Surgical Procedures**: A non-surgical procedure is one that involves the use of a catheter, needle, biopsy instrument or similar tool to obtain small samples or to inject liquids/medications without disruption of function to an animal or is non-invasive in nature. They may be performed with or without anesthesia.
* Examples of non-surgical invasive procedures include, but are not limited to:
	+ Tail snips and ear punches
	+ Percutaneous catheter placements
	+ Cell injections
	+ Blood draws
* Example of non-surgical, non-invasive procedures include, but are not limited to:
	+ Tumor volume measurements
	+ Motor and behavioral studies
* Note: Clipping of hair/fur, use of a surgical scrub, and aseptic technique are still required for procedures.
* Describe all procedures involving the animals used.

Be sure to indicate what specimens are to be taken or harvested, and how this will be done. Also state what you will be testing and/or how you plan to evaluate the results of the purposed research. If planning to draw blood samples indicate how you will obtain the blood, how much will be obtained, and how often it will be collected. Keep in mind that on smaller animals there are limits that must be observed as to how much blood can be drawn at any one time, and how often it can be drawn.

**Aseptic technique** is used to reduce microbial contamination to the lowest possible practical level. No procedure, piece of equipment, or germicide alone can achieve that objective: aseptic technique requires the input and cooperation of everyone who enters the surgery area. The contribution and importance of each practice varies with the procedure. Regardless of the species, aseptic technique includes preparation of the patient, such as hair or feather removal and disinfection of the operative site; preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile surgical gloves; sterilization of instruments, supplies, and implanted materials; and the use of operative techniques to reduce the likelihood of infection. (Guide, 118)

1. **Euthanasia and Final Fate of Animal**
* Differentiate between euthanasia as part of the experimental design and euthanasia required due to illness or injury.

Describe how you will monitor the animals to determine their health status.

* AVMA Guidelines on Euthanasia

Methods of euthanasia must conform to the [2020 Edition of the AVMA Guidelines for the Euthanasia of Animals.](https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf)

* Appropriate methods of euthanasia must always be used

Note that cervical dislocation of mice without anesthesia is not to be utilized without scientific justification. A clear and comprehensive justification must be provided if you are proposing to use this method.

Do not refer to asphyxiation by carbon dioxide as the method of euthanasia. Instead, this should be described as inhalation of carbon dioxide. Note that pre-filled chambers are not appropriate for carbon dioxide inhalation. When inhalation of carbon dioxide is used as a primary method of euthanasia, a secondary physical method such as bilateral pneumothorax, decapitations, etc., must also be used to ensure animal is deceased prior to disposal, and should be included on the AUP.

1. **Animal Supply and Husbandry**
* Housing conditions must conform those found in 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) (8th edition). Any proposed deviations from the Guide must include a justification.

To request CLAF or ENRC study space, PIs must contact the CLAF Manager at claf@uark.edu. Space is limited and is dependent on availability. Each request must be associated with an approved AUP and is subject to a congruency check.

1. **Personnel**
* Include all personnel who will be handling animals, what roles they will have, their qualifications to perform those roles, and any training they have had or will have prior to handling animals.

Training of personnel is a critical aspect of animal care. Students must be properly trained by approved personnel, complete facility (CLAF/ENRC orientations) and adhere to animal care facility procedures. Competency may need to be assessed by animal care staff if personnel do not have experience with a procedure, or if new experimental procedures are being proposed.

## Guidelines and regulations for protocol development

[Guide of the Care and Use of Vertebrate Animals, Eighth Edition](https://olaw.nih.gov/sites/default/files/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf)

[Public Health Service Policy on Humane Care and Use of Laboratory Animals](https://olaw.nih.gov/sites/default/files/PHSPolicyLabAnimals.pdf)

[Animal Welfare Act and Regulations](https://www.aphis.usda.gov/animal_welfare/downloads/bluebook-ac-awa.pdf) – not applicable to birds, rats, mice and poikilothermic species.

[AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)

[Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research](http://www.mammalsociety.org/uploads/Sikes%20et%20al%202011.pdf), American Society of Mammalogists, 2011

[Guidelines to the Use of Wild Birds in Research](https://birdnet.org/info-for-ornithologists/guidelines-to-the-use-of-wild-birds-in-research/guidelines-english-3rd-edition-2010/), The Ornithological Council, Washington, DC, 3rd ed., 2010

[Guidelines for the Use of Live Amphibians and Reptiles in Field and Laboratory Research](http://www.asih.org/sites/default/files/documents/resources/guidelinesherpsresearch2004.pdf). Joint publication of the American Society of Ichthyologists and Herpetologists, The Herpetologists' League, and Society for the Study of Amphibians and Reptiles. 2nd Ed., 2004.

[Guidelines for the Use of Fishes in Research](https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf). Joint publication of the American Society of Ichthyologists and Herpetologists, American Fisheries Society, and American Institute of Fisheries Research Biologists. Fisheries, Vol. 13, No. 2, pp. 16-23, 1988