

I. SECTION I. GENERAL INFORMATION

A. What is an Animal Use Protocol (AUP)?

The AUP describes all uses of live vertebrate animals proposed by an individual Principal Investigator for a specified time period. The AUP presents a description of all proposed animal species, numbers and procedures to be used during the approval period. The Protocol must be fully approved by the IACUC before acquisition, housing or use of animals. Once a Protocol is approved, proposed changes (such as modifications in ongoing procedures, species, or numbers) can be made by submitting a revised AUP. Each investigator is assigned an AUP number that can be used when certifying new grant applications. As used in this application packet, the term AUP will mean a document that describes all proposed uses of animal for the approval period.

B. Who Must Submit an AUP?

Each Principal Investigator who uses live vertebrate animals in research or teaching must submit an AUP. For continuing projects, the AUP must be updated and reviewed annually. Field projects that are strictly observational (i.e., involve no direct handling of animals and no significant disruptions of their environments) need not be included in the AUP. All other off-campus projects, including those in other countries, must be described in the AUP.

C. What is the Approval Period for an AUP?

AUPs ordinarily are approved for a one to five year period. Submission of an AUP for ongoing projects is the responsibility of the investigator. If the project will continue with no changes, renewal may be requested by checking the "no change" box and returning the renewal notice to the IACUC. Changes in personnel and/or anticipated animal housing needs can be accommodated by noting the changes on the renewal form. If changes in animal species, numbers, or procedures are proposed, a revised AUP must be submitted to the IACUC for review.

D. How Can I Modify an Approved AUP?

Once an AUP has been approved, changes in animal species, numbers, procedures, or the location of an activity may require submission of a revised AUP, with the changes shown in colored, bold, or underlined text on the original AUP. Minor changes (such as the addition of a related species or procedure) can usually be administratively approved within several days of receipt. Significant changes must be reviewed by the full membership of the IACUC at the next regularly scheduled meeting. Call or email the IACUC committee for guidance on making changes to your approved AUP.

E. How will extramural funding agencies be notified that animal procedures in a grant application have been approved by the ACUC?

NIH, NSF, and many private extramural funding agencies require verification that the IACUC has approved all proposed animal care and use procedures before they will review or fund research grant applications. When a grant application that involves the use of live vertebrate animals is submitted to the Sponsored Projects Office (SPO), a copy of the application is

forwarded to the IACUC. The IACUC will screen the grant application to see if all proposed animal care and use procedures are described in the investigator's current AUP. If so, the IACUC will notify the funding agency that these procedures have been reviewed and approved. If not, the investigator will be asked to submit a revised AUP describing any new animal care or use procedures.

F. What is the Timetable for Reviewing and Approving an AUP or Changes to an AUP?

NIH Applications: NIH requires verification that an applicant requesting funds for animal research has an approved AUP. The NIH has changed the PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy) to permit institutions with PHS Animal Welfare Assurances to submit verification of IACUC approval for competing applications subsequent to peer review but prior to award. However, it remains incumbent upon investigators to be totally forthcoming and timely in conveying to the IACUC any modifications related to project scope and animal usage that may result from the NIH review and award process. To allow sufficient time for review, AUPs or revisions associated with NIH applications should be submitted to the IACUC as soon as possible. Institutional officials retain the discretion to require IACUC approval prior to peer review in certain circumstances of their choosing if they so desire.

NSF Applications: Because NSF does not require verification of ACUC approval until an award is made, an AUP or AUP revisions associated with an NSF project may be submitted to the IACUC after the sponsor's deadline.

G. How do I Arrange to Acquire Animals?

Live vertebrate animals cannot be purchased or otherwise acquired without an approved AUP. Details on the acquisition and transportation of live animals, including wild animals, should be included in the AUP. The investigator is responsible for determining if permits (such as U.S. Fish and Wildlife or California Fish and Game) are required, and all applicable permits must be obtained before animals are acquired. All permits, such as importation and collection permits, are the responsibility of the investigator to obtain.

II. SECTION II. GENERAL INSTRUCTIONS FOR COMPLETING THE FORM

The following are detailed instructions for completing the Animal Use Protocol Form for Research or Research. Items # 1-9 and Attachments A-D are to be completed on the forms themselves; items # 10-13 are to be answered in narrative form by inserting text in the appropriate sections.

1A. Principal Investigator (PI): (please provide necessary information).

1B. Alternate Principal Investigator (PI): The Alternate PI is someone who can act in the PI's place in the event the IACUC or OLAC is unable to reach the PI for an important time-sensitive decision about the proposed research or a veterinary care issue. The Alternate PI need not have his or her own Master Animal Use Protocol, but he or she will have the designated authority to speak and/or sign for the PI in the PI's absence. PIs should take care to pick an Alternate PI who

is both comfortable assuming this responsibility and likely to be reached at times when the PI is not.

1C. Alternate Laboratory Contact: The alternate laboratory contact should be someone who will be directly involved in the proposed research and who will be capable of answering routine questions about it for the IACUC in the event the PI is temporarily unavailable.

2. General Information: (self-explanatory).

3. Hazardous Agents: Prior approval must be obtained from the appropriate campus safety committee for the use of radioisotopes, regulated carcinogens, biohazards classified at or above biosafety level 2, and NIH non-exempt recombinant DNA. For further information about campus safety committees or any other aspect of hazardous agent use, call the [xx who? contact info]

4. Animal Species, Numbers and Source: Enter the scientific and common name of all species to be used. Indicate the estimated number of each to be used per year. Family or other higher taxonomic level may be used where appropriate. If it is difficult to predict exact numbers or species, give reasonable ranges or examples, respectively. If the project will involve in-house breeding, include breeding pairs and projected offspring in estimate of total numbers. Indicate the source of each species to be used (e.g., commercial vendor, another investigator or institution, wild-caught, etc.). (add more lines as necessary on the AUP)

5. Permits: If wild animals are to be used, the investigator is responsible for determining if permits (such as US Fish and Wildlife or California Fish and Game) are required. Any necessary permits must be obtained before animals are acquired. All other permits, such as importation and collection permits, are the responsibility of the investigator to obtain. (add more lines as necessary on the AUP)

6. Location of Animal Procedures: List all buildings and room numbers where animal procedures will be performed. For field projects, note the location of the field site or facility. (add more lines as necessary on the AUP)

7. Extramural Scientific Review: If the proposed research will not undergo extramural scientific review, in accordance with campus policy, internal departmental peer review may be requested at the discretion of the IACUC.

8. Public Disclosure: Requests for exemption from public disclosure require case-by-case review by the IACUC in consultation with the Principal Investigator.

9. Applicant's Certification: (Self-explanatory)

10. Research Goals: Using non-technical language, briefly describe the goals of the proposed research. Include the potential relevance of the research to human or animal health, the advancement of knowledge, or the good of the society. Please note that your protocol will be returned for revision if this section is not understandable to the non-scientists on the IACUC.

11. Justification for Animal Use: Federal regulations require consideration of the use of alternatives to procedures that may cause more than momentary or slight pain or distress to animals. Alternatives include the following concepts:

- Replacement of vertebrate animals with in vitro models, computer models or less sentient animals;
- Refinement of experimental procedures to minimize pain or distress (e.g., early endpoints, use of analgesics, anesthetics or sedatives; techniques that reduce stress in the animal); or
- Reduction in the number of animals by using appropriate statistical methods in the design and analysis of the study, reduction in variability by using animals of defined genetic or microbiological status, and maximizing the data gained from an individual animal.

11A. Rationale for Use of Animals: Explain how alternatives were considered in the design of the study. Describe why there are no suitable alternatives to the use of live vertebrate animals for accomplishing some or all of the goals of this project. If you already employ, or if you plan to employ, any alternatives to the use of live animals in your research, briefly describe them.

11B. Rationale for Choice of Species: Justify the choice of the species to be studied. If wild animals will be used, indicate the population status of the species or taxa (e.g., abundant, rare, threatened) and the anticipated impact of experimental manipulation and/or collection on the local population.

11C. Rationale for Numbers of Animals: Animal use must be kept to the minimum consistent with a sound scientific outcome. Justify the number of animals proposed for use. List the numbers of control and experimental groups and justify any planned replicates. Explain the proposed group sizes, based on statistical modeling, previous experiments, literature review, or any other method that justifies the numbers of animals proposed. In cases where statistical significance is not expected (e.g., breeding colonies, teaching labs, pilot studies, antibody production, tissue harvesting, etc.) explain why the proposed numbers of animals are necessary. If the protocol includes a number of separate experiments or has a complex design, a table often helps the IACUC better understand total numbers of animals needed.

12. Description of Laboratory Research

12A. Description of Proposed Procedures: Provide a brief description of each procedure to be performed on animals. Include the species and numbers of animals that will be subjected to the procedure, and the duration of the procedure. Provide the following information where applicable:

1) Drug administration: If drugs will be administered for immobilization, tranquilization, anesthesia, analgesia, prophylaxis, or experimental treatment, list for each drug the generic name, dose, route of administration and frequency of administration. If a drug will be administered for experimental treatment, list any potential adverse effects of the drug and describe how animals will be monitored for these effects under item 12.A.3 below, "Pain and Distress."

2) Surgery: If surgical procedures will be performed, provide the information requested below where applicable. For pertinent requirements, see Chapter 3 of the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals (pages 56-70; a copy of the Guide may be obtained by contacting the IACUC office) or consult with a veterinarian.

- Provide a detailed description of all surgical procedures.
- State whether surgery is survival (i.e., recovery from anesthesia) or non-survival. If any intra-operative or post-operative mortality is expected, indicate the anticipated rate and cause.
- Describe the aseptic techniques that will be used in surgical procedures, including how the skin will be prepared, attire of surgical personnel, whether sterile gloves and instruments

will be used, and the method of sterilizing instruments.

- Describe the method for assessing the level of anesthesia, and the frequency of this assessment.
- Describe the methods for preventing dehydration and hypothermia during surgical procedures.
- Describe how post-surgical animals will be monitored and cared for, including who will perform the monitoring, how often it will be performed (including weekends and holidays if applicable), and what criteria will be used to assess an animal's condition. Describe how post-operative pain will be evaluated and alleviated under item 12.A.3 below, "Pain and Distress."
- Provide justification if a single animal will be subjected to more than one major survival surgery. Major surgery is defined as any surgical intervention that penetrates and exposes a body cavity or has the potential for producing a permanent disability.

3) Pain and Distress: If a procedure will induce a disease or deficiency state, or will cause pain, distress or discomfort greater than that associated with routine injections, blood sampling or induction of anesthesia, provide the information requested below where applicable. Please note that survival surgery under anesthesia is considered a painful procedure since animals may reasonably be expected to experience some post-operative discomfort even if analgesics are administered. The following information must be provided for all potentially painful procedures:

- Describe any potential for pain, distress or discomfort to animals. Indicate the likelihood and severity of this pain, distress or discomfort. Be sure to include any potential adverse effects of experimental procedures on animals (e.g., post-operative pain; reduced growth; fever, anemia, or other clinical symptoms of acute or chronic illness or nutritional deficiency; neurological deficits; adverse side effects of experimental drugs; behavioral abnormalities).
- Describe how animals will be monitored for pain, distress, or other adverse effects. Include who will perform the monitoring, how often it will be performed (including on weekends), and what criteria will be used to assess an animal's condition.
- Specify the criteria that will be used to determine when an animal will be removed from the study and/or euthanized prior to the experimental endpoint to prevent undue pain or distress (e.g., x weight loss over y days; a fever exceeding x degrees; an RBC count of less than x; a tumor size of x; failure to eat; failure to groom or huddled position in rodents).

Also provide the following information where applicable:

- If electrical stimulation will be used, describe the methodology and justify the amounts (amplitude, duration and frequency) proposed.
- Provide justification if drugs or other measures will not be used to alleviate pain, discomfort, distress, disease or a deficiency state. Describe documented or presumed interference of alleviating drugs or other measures with experimental results.

4) Food/Water Restriction: If procedures will involve food or water restriction (other than standard pre-operative fasting):

- Justify the need for the restriction.
- Indicate the degree and duration of the restriction.
- Describe any potential adverse effects of the restriction, how animals will be monitored for these effects, and the criteria that will be used to remove an animal from the study to prevent undue distress (e.g., x weight loss over y days).

5) Restraint: If unanesthetized animals will be subjected to periods of physical restraint lasting more than a few minutes, justify the need for restraint, indicate the method, frequency, and duration of the restraint, and describe procedures for conditioning animals to the restraint device.

6) Immunological Procedures: Please contact the IACUC for detailed information on guidelines for immunological procedures.

12B. Method of Euthanasia: Describe any euthanasia method to be used. For recommended euthanasia methods, contact the IACUC veterinarian or consult the current AVMA guidelines. For pharmacologic methods, give generic name, dose and route of administration of the drug. Indicate measures that will be taken to ensure that animals do not revive (e.g., thoracotomy, cardiomy, cervical dislocation). For physical methods, indicate the method to be used and explain why pharmacologic methods are not suitable. When completing Attachment C, "Qualifications of Personnel," be sure to list individuals who will perform physical euthanasia and describe their relevant training and experience.

Other Disposition of Animals: If animals will not be euthanized, describe their ultimate disposition.

12C. Proposed Animal Housing: indicate the location and number of animals to be housed at any one time, and the maximum length of time any individual animal will be kept at that location. In addition, provide the information about animal husbandry requested under item 12E below, "Special Animal Care Requirements."

12D. Special Animal Care Requirements: describe the following where applicable:

- Housing enclosures (size, design, construction materials).
- Number of animals to be housed per enclosure.
- Environmental controls (air or water temperatures).
- Animal husbandry procedures (diet, bedding, water, frequency and methods of cleaning).
- Name(s) and relevant experience of the individual(s) responsible for animal care.
- How often animals will be monitored, and by whom.

12E. Breeding: If project will involve in-house breeding, provide the information requested below. Also describe any special animal care requirements (e.g., nonstandard bedding, less frequent cage cleaning) under item 12.D above, "Special Animal Care Requirements."

- Rationale for breeding rather than using commercial sources.
- Approximate number of colony-bred animals needed for experimental use per project.
- Approximate number of offspring that will be produced to meet that need. If the number of offspring produced will significantly exceed the number needed for experimental use, explain.
- Age at which offspring will be weaned.
- Ultimate disposition of surplus offspring, if any (e.g., transfer to other investigators, euthanasia).
- Approximate number of breeding pairs to be used per project.
- Ultimate disposition of retired breeders (e.g., experimental use, euthanasia).
- Name of person(s) responsible for supervising breeding and weaning.
- How census records will be maintained (e.g., for USDA annual report).

12F. Transportation of Animals: All transportation of animals must be approved by the IACUC. This includes the transport of animals between buildings on campus, and to and from off-campus locations. If your protocol requires transport of animals, indicate:

- Total number of animals to be transported per project.
- Frequency of transport.
- Number of animals to be transported at any one time.
- Transport containers (size, design, construction materials).
- Number of animals to be housed per container.
- How food and water will be provided during transport.

- Who will transport the animals.
- Method of transport.
- Total transport time.
- Precautions that will be taken to avoid subjecting the animals to temperature extremes during transit.

13. Description of Field Research

13A. Animal Capture: Describe animal capture methods, providing the specific information requested below where applicable. For each capture method, list the names, training and relevant experience of the individual(s) who will use that method on Attachment C, Qualifications of Personnel.”

1) Livetrapping: Specify the: a) type of trap to be used; b) time(s) of the year animals will be trapped; c) time of day traps will be set and closed; d) frequency of checking traps; e) whether food, water and/or bedding will be provided in traps; f) precautions to protect trapped animals from extreme heat, cold or rain; g) anticipated rate of injury, if any; and h) method of euthanizing injured animals.

2) Netting: Specify the: a) type of net to be used; b) time of day nets will be set and closed; c) how nets will be monitored after setting; d) frequency of removing captured animals; e) anticipated rate of injury, if any; and f) method of euthanizing injured animals.

3) Darting with Pharmacologic Agent: Specify the: a) type of darting equipment to be used; b) name, dose and site of administration of pharmacologic agent; c) approximate time from darting to capture of animal; d) how animal will be monitored after darting; e) anticipated complications (e.g., escape of animals, capture myopathy, drug overdoses); f) anticipated rate of injury, if any; and g) method of euthanizing injured animals.

4) Other Live Capture Methods (e.g., electroshocking, hand capture): Describe the: a) method to be used; b) the season and the time of day animals will be captured; and c) any potential adverse effects on target and non-target animals, and how these will be mitigated. Include anticipated rate of injury, if any, and method of euthanizing injured animals.

5) Killtrapping: Specify the: a) type of trap to be used; b) time of day traps will be set and closed; c) frequency of checking the traps; d) anticipated mortality rate; e) method of euthanizing injured animals; and f) justification if animals with dependent offspring will be collected.

6) Shooting: Specify the: a) type of firearm and ammunition to be used; b) anticipated escape rates; c) method of euthanizing wounded animals; d) precautions to avoid injuring non-target animals; and e) justification if animals with dependent offspring will be collected.

13B. Animal Restraint/Handling: Briefly describe: 1) Any physical or chemical methods that will be used to restrain or handle animals; 2) Duration of the restraint/handling; 3) Precautions that will be taken to avoid injury or stress to animals.

13C. Animal Marking and Radiotelemetry.

1) Marking: Briefly describe: a) any method used to mark animals; b) the potential for the marking method to adversely effect animal behavior, locomotion, or survival; and c) whether identification markers will be removed at the end of the study. In addition, provide the following information where applicable:

- Toe-clipping: If toe-clipping is proposed, justify why other methods are not suitable, and specify the location and maximum number of toes that will be clipped per animal.
- Bands and Collars: If bands or collars will be placed on young animals, explain the precautions that will be taken to ensure that they do not become too tight as the animal grows.

2) Radiotelemetry: If animals will be tracked using radiotransmitters, describe: a) the method of attaching the transmitter; b) the ratio of the transmitter's weight to the animal's body weight; c) any potential adverse effects of transmitters on animal behavior, locomotion or survival; and d) whether the transmitter will be removed at the end of the study. If surgery is required to implant radiotransmitters, provide the information requested under item 12A above for Laboratory Research, "Description of Proposed Procedures – Surgery".

13D. Description of Proposed Procedures: If animals will only be captured, marked and released, continue to item 13E below. If additional procedures will be performed on animals, provide applicable information requested under item 12A above for Laboratory Research, "Description of Proposed Procedures".

13E. Release, Euthanasia, or Other Disposition of Animals.

1) Release of Animals: If animals will be released into the wild, indicate: a) the proposed site of release and b) how long after capture the animals will be released. If animals will be released after a period of captivity, indicate c) the rationale for releasing animals to the wild; d) any anticipated adverse effects of prior captivity on animal behavior, survival or social status and how these will be mitigated; and e) any potential adverse effects on the local population (e.g., disease transmission) from re-introduction of experimental animals, and how these will be mitigated.

2) Euthanasia: If animals will be euthanized, describe the method to be used. For each method, provide the specific information requested under item 12B above for Laboratory Research, "Method of Euthanasia".

3) Other Disposition of Animals: For other methods of animal disposition, provide the information requested under item 12B above for Laboratory Research, "Other Disposition of Animals".

13F. Recapture of Animals: If animals will be recaptured, indicate: 1) the maximum number of times an individual animal may be recaptured; 2) the intervals between captures; and 3) how many times recaptured animals will be subjected to the procedures described in A-D above.

13G. Health Precautions for Personnel: Briefly describe: 1) any potential health risks to personnel from handling wild animals (e.g., physical injury, arthropod-borne or other zoonotic diseases) and 2) the precautions that will be taken to minimize these risks.

13H. Housing of Captive Wild Animals: If captive animals will be housed at field sites or at other locations off the UNI campus, provide the following information: 1) name and location of the housing site; 2) a detailed description of housing enclosures (size, design, construction materials); 3) the number of animals to be housed per enclosure; 4) means for protecting animals from environmental extremes; 5) a detailed description of animal husbandry procedures (diet, bedding, water source, frequency and methods of cleaning); 6) the name(s) of the individual(s) responsible for animal care; 7) how often animals will be monitored, and by whom; 8) the name, title and telephone number of the site manager; and 9) whether approval has been granted for use of the site.

13I. Transportation of Animals: If animals will be transported from the site of capture, briefly describe: 1) who will transport animals; 2) transport containers (size, design, and construction materials); 3) the number of animals housed per container; 4) how food and water will be

provided during transport; 5) method of transport; 6) total transit time; and 7) precautions that will be taken to avoid subjecting the animals to temperature extremes during transit.

14. Description of Teaching or Educational Use.

14A. (self-explanatory)

14B. Educational Goals: Using non-technical language, briefly describe the educational goals of the proposed animal use. What are the general objectives of the classroom exercise? Make sure that you have also completed section 11, "Justification for animal use", above, as it applies to teaching.

14C through 14H: see Instructions for 12A through 12F, under "Description of Laboratory Research".

Attachment A: Methods and Sources Used to Consider Alternatives to Potentially Painful Procedures.

USDA regulations require the IACUC to ensure that: "The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, e.g., the Animal Welfare Information Center, used to determine that alternatives were not available." (9CFR §2.31,d,ii)

Many investigators satisfy this requirement by describing a literature search. A useful source of information on techniques for conducting such a search, and list of possible databases to be searched, is found at the website for the Animal Welfare Information Center, or AWIC, (<http://www.nal.usda.gov/awic/>). If a literature search is cited, USDA Policy 12 (<http://www.aphis.usda.gov/ac/policy/policy12>) requires that the narrative must include the:

- names of the databases or sources consulted
- date of the search
- years covered by the search
- key words or search strategy used
- description of search results and applicability to your proposed research

Methods other than a literature search may include peer review of your research, and evidence that you are knowledgeable in your field of research. Such items include:

- years of relevant experience
- list of professional conferences/meetings attended
- names of other experts consulted
- list of service provided to grant review committees, panels or editorial boards
- list of journals subscribed to and/or read
- list of seminars/lab meetings presented/attended

Whatever methods are cited, the IACUC must be convinced that a good-faith effort was made to identify and consider alternatives to any potentially painful procedures.

Attachment B: Non-Duplication of Research.

Federal regulations (9CFR §2.31,d,iii) also require assurance from the PI that the work proposed in this protocol does not unnecessarily duplicate previous experiments. Provide a statement indicating that studies such as these have not been done, and indicating the sources used to make that determination (e.g., database search, attendance at meetings, conversations with colleagues). You may refer to the search above (Attachment A) if appropriate. If the proposed studies are similar to those done previously, please provide a justification for the need to duplicate this earlier research.

Attachment C: Qualifications of Personnel.

List the names, titles, phone numbers, and email addresses of all known personnel who will work directly with animals. For each person listed, indicate the species and procedures to be used, and briefly describe the individual's relevant training (how and by whom they were trained) and experience. Include the PI and anyone other than the PI who will directly supervise students and/or research staff.

U.S. Animal Welfare Act Regulations (AWAR) and Public Health Service (PHS) Policy require institutions to provide training for all personnel engaged in animal research. This includes Principal Investigators, technicians, laboratory personnel, research fellows, students, and visiting scientists. Even PIs who do not, themselves, work directly with animals are required to have general training in animal care and use because of their ultimate responsibility for the personnel listed on their animal use protocols. The content of this general training is specified by federal regulations and policy. The IACUC has established the following policy on training requirements for animal users.

- Prior to the start of any animal activity, all faculty, students and research personnel who use live vertebrate animals in research or instruction must receive training as specified by the AWAR and PHS Policy.
- It is the responsibility of the PI to ensure that all personnel listed on the PI's animal use protocol receive the required training in animal care and use. This training, which must be documented in writing, may be obtained directly from the PI or by attending a training seminar. If the PI provides this mandated training, an outline of the topics covered must be submitted to the IACUC to ensure compliance with federal regulations and policy.