Animal Research and Teaching Protocol Form

Instructions for Completing Animal Research and Teaching Protocol Form

Form must be typed.

Any research conducted by a student that involves humans and/or animals requires the approval of ORSP: Human and Animal Protections. Such approval must be obtained prior to the initiation of any research activity involving these subjects. There are no exceptions to this required approval.

A student whose work involves humans or animals is not permitted to enroll in the Culminating Experience course until the research activity has been approved by the Human and Animal Protections Committee. Students should check with the Human and Animal Protections web site for specific information (http://research.sfsu.edu/protocol/).

Form Begins on Page 2

PLEASE NOTE: in order save your personal information on the following PDF forms, you will need to:

2. Save the PDF form to your computer desktop prior to entering your personal information.
Animal Subjects Protocol Form and Instructions

This section contains the forms needed to obtain Institutional Animal Care and Use Committee (IACUC) review and approval of research and teaching projects using live, vertebrate, non-human animals, as required by federal law and SFSU policy.

Regulations require that:

- The IACUC reviews all animal use carried out in university facilities, as well as fieldwork and research at other institutions conducted by SFSU personnel. All projects are to be approved prior to the actual use of animals, whether it involves research/teaching or warm/cold blooded vertebrates or vertebrate animal products.

- The approval of animal use will be granted for a three-year period for non-USDA regulated species and one year for USDA regulated species (e.g., rabbits, guinea pigs). The IACUC is required to review activities annually, even though it may be a multiple-year project.

The forms are designed to help provide sufficient information to allow a meaningful review of your proposed animal use. To obtain project approval, please follow these instructions:

- Provide a TYPED copy of your completed Animal Subjects Form. Handwritten copies will be returned without review.

- Complete all information and be explicit, but brief, when providing details. "See attached proposal" is not an acceptable response. Forms submitted with inadequate information will be returned.

- Attach a copy of the grant application, proposal, or other documents giving detailed descriptions of all procedures involving animals.

- All researchers are required to pass the SFSU online animal research certification course (or its equivalent from other institutions), which takes approximately 2 hours. A certificate is issued to the researcher upon successful completion of the course. The certificate must be printed out and included in the protocol materials. Researchers must provide a copy of the certificate with each protocol they submit. The certificate is valid for three years from the date of certification.

Be advised that the Animal Subjects Form is a public record and may be released upon request. Should certain information be confidential (e.g., experimental design/hypothesis, materials used, etc.), please indicate such items.

Sufficient lead-time is necessary to obtain approval and should be taken into consideration in planning start dates or need for approval for funding agencies. Please allow 2 months for UACUC review and decision.

Assistance for animal procedures and use of anesthetic/analgesic procedures and methods of euthanasia is available (see appendix for euthanasia guidelines). The attending veterinarian must be consulted in the planning of potentially painful/stressful procedures. Should you have questions regarding specific items on the form or general questions involving animal regulations/policies, contact the UACUC administrator at 415-338-1093 or protocol@sfsu.edu.
ANIMAL RESEARCH AND TEACHING PROTOCOL FORM
San Francisco State University

A completed protocol form must be submitted for review to the SFSU Institutional Animal Care and Use Committee (IACUC), c/o GradStop ADM 250. Allow at least eight weeks for the review process.

Title of Project: ____________________________________________

Principal Investigator: __________________________ Email: __________________

Address: ___________________________ Department: __________________

________________________________________ Telephone: __________________

Rank: □ Undergraduate □ Master □ Faculty □ Other (describe): __________________

If for a course, indicate course # __________________________

Anticipated Beginning and Ending Dates: ___________ to ___________

mo / dy / yr mo / dy / yr

Faculty Advisor/Sponsor Assurance Statement:

As the Faculty Advisor/Sponsor, I certify that I have reviewed this protocol and affirm the merit of this research project and the competency of the investigator(s) to conduct the project. (A signature is required for all student research projects, undergraduate or graduate, and for all persons not affiliated with SFSU.)

_________________________ _________________________
Name Signature Email Date

Department Chair, Graduate Coordinator, or Designated Colleague:

_________________________ _________________________
Name Signature Email Date

For Office Use Only

Approved □ Not Approved □

_________________________ _________________________
Chair of IACUC Approval Date
Field Observation Project

If the animal procedures planned for this research involve simple field observations in which the lay public could also participate with NO IMPACT ON EITHER THE ANIMALS OR THEIR ENVIRONMENT, it is not necessary to complete the subsequent sections of this protocol form unless permits are required. Instead:

- On a separate sheet of paper describe the study activities and goals of this activity. Include all the precautions taken to ensure no adverse impact on the study animals and their environment.
- Provide documentation that permits and/or letters of permission are not required.
- Sign the Assurance form.
- Complete the SFSU Online Animal Research Certification Course.

Use of Existing Specimens

If the research involves solely the use of museum specimens or specimens that someone else has gathered, or specimens that the researcher previously gathered, it is not necessary to complete the subsequent sections of this protocol form. Instead:

- On a separate sheet of paper describe the study activities and goals of this activity.
- Provide a letter from the source authorizing access to the samples and indicating that samples were collected appropriately.
- Sign the Assurance form.
- Complete the SFSU Online Animal Research Certification Course.

Use of Avian Embryos

Research involving avian embryos may not require a complete protocol. Please see the Policy for Use of Avian Embryos to determine if your research requires a complete protocol. If your research does not require a complete protocol, it is not necessary to complete the subsequent sections of this protocol form. Instead:

- Complete the Avian Embryo Use Summary form attached to the Policy for Use of Avian Embryos.
- Sign the Assurance form.
- Since you are not considered to be using live animals, you do not need to complete the SFSU Online Animal Research Certification Course.

All Other Research

1. Animal Species, Numbers, and Sources.
   List all animal species (and strain) and indicate numbers to be used per year. Give common name for standard laboratory species, and both common and scientific name for
non-standard laboratory species. Indicate the source of each species to be used. Attach additional sheets if necessary.

<table>
<thead>
<tr>
<th>Species (and Strain)</th>
<th>Number per Year</th>
<th>Source</th>
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2. Location of animal procedures and/or housing.
List all locations where animal procedures will be performed. If off-campus, indicate where (e.g., Point Reyes National Seashore, UCSF). (Check with the facility Director before identifying on-campus facilities.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Housing</th>
<th>Surgery/Recovery</th>
<th>Euthanasia</th>
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3. Are permits required for importation, collection or maintenance of animals?

☐ No ☐ Yes. If yes, list agencies that require permits, and permit status below. Submit a copy of the permits to the IACUC with each application.

1. Agency
Permit Application: ☐ Approved ☐ Pending ☐ To be submitted

2. Agency
Permit Application: ☐ Approved ☐ Pending ☐ To be submitted

4. Will any hazardous materials/agents be administered to the animal?

☐ No ☐ Yes. If yes, explain risk and safety procedures to be followed by laboratory and animal facility personnel (for infectious agents also indicate the appropriate CDC biosafety level). Please consult SFSU documents concerning “Biohazard Control, Biological and Carcinogenic Agents.”
5. List below all persons, other than the PI, who will have significant contact with the animals involved. All persons having contact with animals or animal products must complete the animal research certification course.

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Involvement (Co-Investigator, Graduate Assistant, etc.)</th>
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6. Funding Sources and Dates:

    __________________________________________
    __________________________________________
    __________________________________________

Narrative (For survival surgery, also complete the supplemental Survival Surgery Form)

A. Objective/Significance. Describe in non-technical terms, the scientific or educational aims of the project. Justify the project in terms of its potential value in obtaining or establishing significant information relevant to the understanding of humans or animals, maintenance and improvement of human or animal health and welfare, improvement of animal management or production, or achievement of educational objectives.

B. Lay Description of Experimental Design: In language that will be understood by members of the general public, provide a succinct outline of the formal scientific plan and direction for experimentation. If several experimental groups or sequential studies are to be included in the protocol, description of the experimental design for each separate experimental group should be contained in sub-parts of this section.

C. Methods. Describe the experimental protocol in detail. Include information on behavioral, dietary, environmental, pharmacologic, physiologic, surgical, etc., manipulations. Describe procedures designed to minimize discomfort, distress, pain and injury to the animals. If anesthetics, analgesics or tranquilizers are to be used, detail the dosage, route of administration, and duration.

For field research, include details of animal capture, handling, restraint, marking, and release. (While the research design may not include euthanasia, this may be necessary if animals are inadvertently injured. Emergency euthanasia methods need to be identified below.)

D. Euthanasia. Explain the methods to be used and the reasons for their selection. List agents, dosages and routes of administration. Provide information on the final disposition of the animal.
E. Investigator Qualifications. Describe your qualifications and training for carrying out all animal procedures and what training you will provide for persons handling animals. It is your responsibility as the PI to ensure that all personnel handling animals have been appropriately trained. The training must be registered with the IACUC.

F. Animal Use. Justify the use of animals vs. non-animal alternatives, the choice of species (why this is the most appropriate species/strain to use in these studies), and the numbers of animals to be used (provide a breakdown of the animals into experimental group, identifying each experimental group and the numbers of animals in each group).

In discussing alternatives, consider the “3 Rs”:
   a. Replacement: Those methodologies (computer programs, tissue culture techniques, epidemiological data, etc.) which replace the use of animals.
   b. Reduction: Those methodologies which reduce the numbers of animals used in the protocol.
   c. Refinement: Those methodologies which refine the procedure to minimize the amount of discomfort that the animal may experience.

Include the computer database searched (e.g., Medline, Index Medicus, etc.) or other sources, such as journals or meetings that you used to determine that: a) there are no appropriate alternatives for this research and/or b) this protocol does not unnecessarily duplicate previous experiments by yourself or others. A computer search of at least two databases is required. A search for alternatives may include words such as “cell culture”, “in vitro”, or “computer models” or it may include a less sentient animal species. The search should be for reduction and refinement, not just replacement of animals.

Provide the databases searched, the key words used, the years searched, and the date of your search. Summarize the outcome of your search. If there are any hits, explain: a) why these would not be acceptable as a replacement for your in vivo work and/or b) how your study differs from previous work.

Three databases designed specifically to search for alternatives that you might want to use are:

   http://altweb.jhsph.edu/

   http://www.vetmed.ucdavis.edu/Animal_Alternatives/altsearch.htm

G. References/Bibliography
APPLICANT’S ASSURANCE

I assure that:

A. This protocol provides a complete and accurate description of all proposed uses of live vertebrate animals or animal products in this research activity. Any proposed revisions to animal care and use procedures will be promptly forwarded in writing to the IACUC for review.

B. I will abide by all applicable laws, policies and regulations, including the Animal Welfare Act, the NIH Guide for the Care and Use of Laboratory Animals, and all SFSU policies and procedures regulating the humane use of vertebrate animals or animal products in instruction and research. I will ensure that students, fellows and staff under my supervision have access to and are familiar with these policies and will comply with the procedures described.

C. I will comply with all regulations governing the importation, collection and/or maintenance of wild species, including obtaining permits from all applicable regulatory agencies prior to the acquisition of animals.

D. All experiments involving live animals will be performed under my supervision. Listed participants will perform only those procedures for which they have received adequate training.

_________________________  _______________________
Signature  Date
Appendix:
San Francisco State University
Institutional Animal Care and Use Committee Euthanasia Guidelines

Purpose: These guidelines have been developed to provide minimum standards for euthanasia of commonly used laboratory animals and assure that all SFSU procedures involving euthanasia of experimental animals are in compliance with federal animal welfare regulations and follow standards described in the American Veterinary Medical Association (AVMA) Euthanasia Guidelines and the Guide for Care and Use of Laboratory Animals (Eighth edition). It is the responsibility of the investigator to ensure the use of techniques and procedures which result in the least pain and distress to the animal while adequately addressing the needs of the experimental design. All euthanasia methods must be reviewed and approved by the IACUC prior to their implementation. Exceptions to these guidelines are possible if justified for the experiment and reviewed and approved by the IACUC.

Training: It is the responsibility of the investigator to assure that all individuals performing euthanasia are adequately trained to do so. Competency for performing all methods of euthanasia without supervision by a previously certified individual must be certified by the veterinarian or Animal Facility staff. Additional competency for performing euthanasia by decapitation or cervical dislocation without anesthesia must be certified by the veterinarian.

Euthanasia of Rodents

Methods: Anesthetic Overdose
a. Chemical euthanasia with an overdose of intravenous pentobarbital is the euthanasia method preferred by the AVMA for mammals with practical vascular access (e.g. rabbits).
b. Intraperitoneal pentobarbital or an overdose of inhaled anesthetic (e.g. isoflurane) is acceptable for smaller mammals/reptiles/amphibians when intravenous access is not practical.
c. Chemical euthanasia must be followed by a physical procedure that assures death (e.g. bilateral thoracotomy, cervical dislocation, pithing, or organ removal).

Methods: Carbon Dioxide Inhalation
a. Exposure to CO₂ gas in an enclosed chamber is an acceptable form of euthanasia for small mammals with conditions as outlined below.
b. Compressed CO₂ in cylinders is the only allowable source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods, such as from dry ice, is unacceptable. The CO₂ flow rate into the chamber should displace 10-30% of the chamber volume per minute.
c. Animals should be placed in the chamber or container and the lid replaced or closed. The entire home cage of animals should be used without introduction of new animals, whenever possible. Rodents must never be “collected” in a group cage with unfamiliar rodents prior to euthanasia.
d. Unconsciousness will occur within 30 seconds but animals should be left in the container with the gas flowing for at least an additional 60 seconds. Neonates should be exposed for 5 minutes. Never leave a euthanasia chamber with flowing gas unattended.
e. Prior to carcass disposal, a second physical means of euthanasia must be performing to assure death: cervical dislocation (rodents < 200 g), bilateral thoracotomy (rodents > 200 g), decapitation (neonates) or organ removal. The physical euthanasia method may be performed as soon as the animals lose their righting reflex.
Methods: Cervical Dislocation/Decapitation with Guillotine

- The IACUC recommends anesthesia for animals undergoing cervical dislocation/decapitation.
- When properly performed, cervical dislocation may be used for euthanasia of mice and other small rodents and rats weighing less than 200 grams.
- When properly performed, decapitation may be used for euthanasia of mice and rats.
- The following conditions must be met if cervical dislocation/decapitation without pre-anesthesia is performed:
  1. The AUP must document the scientific justification for this request and provide documentation of experience and/or training of the person(s) who will be performing the procedure. Veterinary certification will be required.
  2. If a guillotine is used for decapitation, “the equipment used for decapitation should be maintained in good working order and serviced on a regular schedule to maintain sharpness of blades.” (AVMA Guidelines) Guillotines in use must be sharpened at least annually. If equipment has been out of service it must be sharpened prior to re-commissioning.
  3. Guillotine use and maintenance must be logged and records must be available for review by Animal Facility staff and the IACUC.

Euthanasia of Neonatal Rodents and Fetuses

- Neonates: Euthanasia of rodent neonates is the same procedure as for adults described in the above methods except that AUP approval is not required for decapitation without anesthesia. Neonates of altricial rodents (mice, rats, hamsters) are relatively resistant to the effects of CO2 and other inhalants and may require longer exposure times. A secondary physical method (cervical dislocation, decapitation) is required.
- Fetuses:
  - For mouse, rat and hamster fetuses up to 15 days and guinea pig fetuses up to 35 days of gestation - euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to blood loss and non-viability, thus no additional method of euthanasia is required.
  - For mouse, rat and hamster fetuses >15 days and guinea pig fetuses >35 days of gestation - after general anesthesia or euthanasia of the mother, decapitation or cervical dislocation of the fetus must be performed.

Euthanasia of Non-mammalian Vertebrates including Birds, Reptiles, Amphibians and Fish

The same principles for euthanasia of small mammals apply to birds and ectotherms: a physical method must follow chemical euthanasia and physical euthanasia without anesthesia must be justified in the AUP and approved by IACUC.

Common Methods

1. Chemical Methods
   a. An overdose of pentobarbital can be used to euthanize ectotherms and birds via intracoelomic/intraperitoneal injection. The actual time to death may be very prolonged for ectotherms.
   b. pH-neutralized Tricaine methanesulfonate (MS-222) administered in water may be used to euthanize fish, aquatic and semi-aquatic amphibians. Reptiles may be euthanized by intra-coelomic (IC) injection of 250-500 mg/kg pH-neutralized 1% MS-222, followed by 0.5-1 ml 50% unbuffered MS-222 (Conroy et al, 2009).
c. Benzocaine administered in water may also be used to euthanize fish, aquatic and semi-aquatic amphibians. A minimum 0.03% solution of benzocaine is recommended for euthanasia.

2. Physical Methods
   a. Freezing following cessation of respiration after chemical euthanasia is acceptable. Freezing is NOT allowed as the sole method of euthanasia. Lowering the ambient temperature may facilitate handling; however, there is no evidence that it raises the pain threshold in ectotherms. Formation of ice crystals on the skin and in the tissues of an animal may cause pain and distress.
   b. Decapitation: the central nervous system of reptiles is extremely tolerant to anoxia. Therefore, methods of euthanasia that induce unconsciousness by interruption of blood supply to the head, e.g., decapitation, cervical dislocation, and exsanguination, are inappropriate for reptiles when used alone. These methods can only be used on small reptiles that are already unconscious by a chemical agent or concussion or when followed immediately with double-pithing, freezing in liquid nitrogen or dry ice.
   c. Double-pithing can only be carried out on unconscious animals and performed by trained personnel.

**Birds and Reptiles: Hatchlings, Embryos and Eggs**

1. Hatchlings
   a. Avian and reptile embryos that hatch, either intentionally or unintentionally, are live vertebrate animals and are regulated by PHS Policy. For this reason, any project in which birds or reptiles may hatch must include a contingency plan for humane euthanasia of hatchlings in the MAUP.
   b. Acceptable methods of euthanasia of avian or reptile hatchlings include overdose of CO₂ or anesthetic agents. A secondary physical method is required to ensure death, for example decapitation, cervical dislocation (birds) or decapitation/pithing (reptiles).

2. Embryos & Eggs
   a. For embryos/eggs >50% gestation, use methods appropriate for hatched birds or reptiles (see above).
   b. For embryos/eggs <50% gestation, prior to neural tube formation (Close et al, AAZV guidelines), destroy the viability of the egg via one of the following methods: shaking, puncturing, freezing (<4°C for 4 hours) or coating eggs with oil.

**Amphibians and Fish: Larvae and Embryos**

1. Larvae: For the purposes of these guidelines, the larval stage of amphibians and fish begins at the same time as hatching and independent feeding. Acceptable methods of euthanasia for adult amphibians and fish apply to the larvae of these species (i.e., anesthetic agent followed by secondary physical method).
2. Embryos: For the purposes of these guidelines, amphibians and fish are embryos prior to the larval stage (i.e., up to hatching and independent feeding), and therefore have yet to develop pain systems (AHAW Panel Report). Embryos of these species are disposed of depending on their experimental treatment and transgenic status, but care should be taken to recognize the variation among these species of the stage at which independent feeding begins.
References


Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002.


Report of the ACLAM task force on rodent euthanasia, Toth et al., October 2005.