**Instructions for completing the UC Merced protocol form for Animal Use and Care**
Please make sure to check grammar and spelling prior to submitting application. Mistakes in grammar and spelling in the application can result in misunderstanding of species, procedures, husbandry requirements, etc, by the members of the IACUC committee and IACUC staff.

**1. Contacts**
The investigator must be a UC employee with career status. Non UC personnel, graduate students, and residents may be the alternate contact, but the responsible party must have career status employment. Please be sure to include an alternate contact along with after hours phone and/or pager numbers. If animals on this project show evidence of illness or pain, emergency care, including euthanasia, will be administered at the discretion of veterinary staff when the investigator or alternate contact person cannot be reached.

**2. Title**
Please limit the project title to 60 characters as frequently required for grant applications.

**3. Species**
Please only list species by common names such as mice, rats, dogs, rhesus or cynomolgus monkeys, or cats. List the total number of animals to be used over the three-year period covered by the submitted protocol. If you have a breeding colony producing the animals for this study, then all of the animals in the colony will need to be accounted for in the protocol. If more than three species will be used, please provide an attached list.
Assumed litter sizes: 8-10 mice/rats

**4. Procedures**
Briefly describe the procedures included in this project using language that would be understandable to those without a scientific background. This page is posted on the animal room door for animal care staff, and it is important that they understand the intent of the study. This information will help the animal care staff understand any conditions they may encounter while caring for your animals, and best serve your needs and those of the animals.

**5. Animal Location**
Please list the vivarium location as well as the study area or laboratory where ANY of the procedures described in the protocol will be performed. If animals are to be investigator-maintained, husbandry SOPs must be submitted with the protocol form or must be on file with the IACUC Office.

**6. Special Husbandry Requirements**
Describe any special requirements your animals will have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions associated with husbandry. Be sure to identify any deviations from the Guide for the Care and Use of Laboratory Animals, such as wire bottom cages, and include the length of time animals are to be housed in the special cages. Justification for deviations from the "Guide" must also be provided. Include any special instructions with regard to disposal of dead animals, such as "bag for disposal" or "save for necropsy". Also indicate if pest control can be performed in the animal area. The "Guide" is a document intended to assist institutions in caring for and using animals in ways

judged to be scientifically, technically, and humanely appropriate. The "Guide" is also intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles. It is expected that all "Guide" recommendations will be followed, unless specifically justified.

**7. Hazardous Materials**

The use of Hazardous Materials in the animal rooms and the laboratories requires the completion of the Room/Lab Safety Information sheet found at the end of the protocol form. The use of such agents may also require a Use Authorization from Environmental Health & Safety (EH&S). The Investigator is responsible for insuring that the necessary approvals are obtained from EH&S, that all personnel are appropriately trained and equipped to work safely with these hazards, and that the animal care staff has been notified of any associated risks specific to the hazardous agent(s) and its use in animals. Hazardous materials include: Infectious Agents; Radioisotopes; Chemical Carcinogens; Recombinant DNA; and Hazardous Chemicals (flammable, toxic, corrosive, or chemotherapeutic). For more information regarding specific requirements pertaining to hazardous materials, contact EH&S at (209)228-4234.

**8. Funding and Funding Source**

Please indicate if this is a NEWLY funded NIH grant. The Public Health Service (PHS) policy requires the IACUC to compare the animal portions of a newly funded grant to the submitted protocol. To accomplish this requirement, investigators are requested to submit those portions of the submitted grant that reflect any proposed procedures with live vertebrate animals. Please submit relevant animal-related pages from section D. Experimental Design and Methods. For example, if the grant contains tables describing the study groups and numbers of animals, please include. Please also attach section F. Vertebrate Animals. The pages that are submitted with the protocol as an attachment must be from the submitted grant. Please contact the IACUC staff if you have any questions associated with this section of the protocol submission and the PHS requirements. The IACUC suggests that investigators consider adding relevant information to section F. Vertebrate Animals in new grant applications. This will provide an easy method for including the necessary information for new grant-related protocol submissions.

**9. Veterinary Care**

IACUC Policy on Veterinary Care Delivered by Faculty Veterinarians:
Adequate veterinary care is an institutional responsibility. The Attending Veterinarian, Dr. (?) is the veterinarian of record for the UC Merced campus, as required under the Federal Animal Welfare Act, and has final responsibility to insure that campus programs of veterinary care are adequate. Veterinarian-researchers who wish to directly provide veterinary care for their animals may do so, subject to the following:

- The investigator must demonstrate familiarity with the regulatory requirements for adequate veterinary care.
- At the beginning of the experiment, the veterinarian-researcher must consult at least once with the veterinary staff of the unit in which their animals will be housed. The purpose of this consultation is to work out any unresolved details of veterinary

care, such as weekend coverage when the investigator is out of town, etc.
• The investigator will be required to keep individual medical records in the facility with the animal(s) at all times so that these records are accessible to campus veterinary staff and the IACUC. Animal records must remain with the animal facility when the project is completed.

The designated veterinary staff for the unit retains the responsibility for the adequacy of veterinary care, and retains oversight responsibility for animals within their units. Animal caretakers must continue to report sick calls through the appropriate channels.

**10. Objectives and significance**

Please provide the overall objective(s) of the study, and the significance. Bear in mind your target audience may be a faculty member from an unrelated discipline, please do not use jargon. All abbreviations must be clearly defined.

**11. Literature Search**

A minimum of two database searches that reflects an effort to address the '3Rs' (refinement, replacement, reduction) is required by federal law. It is also important to show that the proposed use of animals is not unnecessarily duplicative of other studies previously performed. The '3Rs' are:

• Refinement of technique to reduce or eliminate unnecessary pain and distress an animal may experience.
• Reduction, which refers to an effort to reduce the number of animals used overall within the study.
• Replacement refers to replacing animals with non-animal alternatives, non-mammalian or invertebrate species. Alternatives could include in vitro methods that utilize organ, tissue and cell culture, computer simulation models, microorganisms, plants, or chemical techniques.

UC Merced provides on-line access to databases that can be used to search for such alternatives. The literature search must have been performed within the last six months. For more information on animal alternatives please visit USDA for guidelines on adequate literature searches for animal alternatives.

Animal Alternative Databases:
UC Center for Animal Alternatives
AWI Laboratory Animals
Animal Welfare Information Center
Center for Alternatives to Animal Testing

**11b. Result of search for alternatives.** Please indicate the results of this search for alternatives, and whether you were able to identify alternatives that are included in the studies proposed. If no alternatives have been found, and if no prior studies have been performed that indicate duplication, then state this within the framework of the objectives of

the study. It is important to state the unique aspects of your study in relation to the published literature. Please also include any procedures you have performed that indicate your efforts to decrease the use of animals. This could include in vitro studies or those performed with invertebrate or non-mammalian species. This documents your efforts towards employing the concepts of the '3Rs'.

**11c. Animal numbers justification.** Please include a description of how you have arrived at the numbers of animals you are requesting for these studies. A rationale for the numbers of animals proposed is essential, and the IACUC is required by law to assess the need for the numbers of animals proposed for use. Statistical validation can be presented using a power analysis or other relevant statistical tests. Such information allows the IACUC to understand your study needs and assure that the scientific objectives of the study can be met (ie; sufficient animals, too few animals or too many animals).
For protocols that do not require statistical significance such as a pilot study, please include a justification for the numbers of animals that includes potential variability that may be anticipated and how that affects the study. Animal number justification is also required for teaching protocols, demonstrations, or for breeding stock. For other studies, such as those that focus on specific cell types or tissue analysis, include a detailed breakdown identifying the amount of material required to accomplish the goals, how much can be obtained from each individual animal, and how this is reflected in the animal numbers requested. This will allow the IACUC to understand your study needs.

**11d. Species rationale**. Please include your rationale for the species chosen and why in vitro methods cannot be used to meet the stated objectives.

**11e. Has this study been previously conducted?** If you are proposing to repeat a study that has been previously conducted, it is necessary to provide justification for this need. The IACUC is required to insure that no unnecessary duplication is performed and your justification will allow the IACUC to understand why duplication is necessary.

**12. Summary of Procedures**

**12a. Describe the use of animals in your project in detail.**

In this section please clearly and succinctly state all of the procedures that you propose to perform with the animals. Please include in your description terminology that will be understood by individual outside of your area of expertise, and only use abbreviations after they have been defined. Please be concise and describe the procedures in a manner that can easily be followed. For complicated experimental designs, a flow chart or diagram is strongly recommended to help the IACUC understand the experiments you propose. Please provide full descriptions of any surgical procedures. If the procedures proposed will be performed by a vivarium veterinarian and is described in an IACUC approved SOP, then it is only necessary to state the title of the SOP and SOP number. See individual vivaria veterinary staff to obtain this information.

Specifically address the following:

- Experimental injections (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules).

• Blood collection (volume, frequency, withdrawal sites, and methodology).
• Surgical procedures (animal prep, approach, procedure, closure and post-op care).
• Radiation (dosage and schedule).
• Methods of restraint (e.g., restraint chairs, collars, vests, harnesses, slings, cones, etc.). Include how the animals are restrained for routine procedures such as blood collection. Prolonged restraint must be justified with appropriate oversight to insure it is minimally distressing. Describe any sedation, acclimation or training to be utilized.
• Experimental endpoint criteria (e.g., tumor size, percent body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptoms, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant clinical symptoms or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be justified. Provide details in sections 12c and 13.
• Field studies: If animals in the wild will be used, describe how they will be observed, any interactions with the animals, whether the animals will be disturbed or affected, and any special procedures anticipated. Indicate if Federal or State permits are required and whether they have been obtained. Please attach copies of permits if applicable.

If your protocol includes antibody production, please include the Anitbody Production Project Description.

**12b. Study Groups and Numbers Table**

As identified by the columns, in this table please include the group number, the procedures for each of the animals in each group, and the number of animals per group. A separate table can be included if this is preferred. This table must fully account for all animals used in the proposed studies, and the total number of animals proposed needs to match the total number of animals shown on page 1, Section 3: Species.

**12c. Death as an Endpoint**

"Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation. The use of death as an endpoint is discouraged and must always be justified. Endpoints other than death must always be considered and should be used whenever the research objective can be attained with non-lethal endpoints. If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, based on defined clinical signs, death is not an endpoint.

**12d. Surgery**

Please identify if the surgical procedure(s) proposed are survival or terminal, the surgery location, and personnel involved. If both survival and terminal procedures are indicated in this protocol, please clarify both groups.

**12e. Multiple Major Surgical Procedures**
Federal Law requires scientific justification for multiple major surgical procedures. Major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function. Multiple major survival surgical procedures on a single animal are discouraged but may be permitted if scientifically justified by the investigator and approved by the IACUC. For example, multiple major survival surgical procedures can be justified if they are related components of a research project, if they will conserve scarce animal resources per the National Research Center 1990, or if they are needed for clinical reasons. If multiple major survival surgery is approved, the IACUC must pay particular attention to animal well-being through evaluation of the outcome(s) of the animals through regular updates provided by the investigator. Cost savings is not an accepted justification for performing multiple major survival surgical procedures.

**12f. Drugs to be used (except for euthanasia) -** Anesthetics, analgesics, tranquilizers, neuromuscular blocking agents, antibiotics and other drugs.
The Animal Welfare Regulations and Public Health Service Policy state that procedures which may cause more than momentary or slight pain or distress to the animals will:

- be performed with appropriate sedatives, analgesics or anesthetics unless withholding such agents is justified for scientific reasons, in writing, by the Principal Investigator and any withholding will continue for only the necessary period of time.
- involve in their planning, consultation with the Attending Veterinarian.
- not include the use of paralytics without anesthesia.

Please provide information about anesthetics, analgesics, tranquilizers, neuromuscular blocking agents and antibiotics according to species, drug, dose, route, and timeline that you intend to use in this project. Please consult with vivaria veterinary staff relevant to the species under consideration to insure drugs and dosages are appropriate.

**12g. Anesthesia Monitoring**
Please describe the physiologic parameters you will monitor during the procedure to assess adequacy of anesthesia and indicate the circumstances when incremental doses of anesthetics will be administered. If vivarium staff are responsible for anesthesia monitoring, please state.

**12h. Neuromuscular Blocking Agents**
Neuromuscular blocking agents can conceal inadequate anesthesia and therefore requires justification. Please describe the physiologic parameters to be monitored while under a neuromuscular blocking agent to assess adequacy of anesthesia.

**12i. Post-Surgical Monitoring**

Please describe the physiologic parameters you will monitor during the recovery period as well as how often these parameters are monitored. Also indicate when post-operative analgesics will be given or provide scientific justification if postoperative analgesics cannot be given. If vivarium staff are responsible for post-procedural monitoring, please state.

**13. Adverse Effects**

Describe all significant adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits, behavioral abnormalities, nutritional deficiency or other clinical symptoms of acute or chronic distress). For genetically altered animals it is essential to include any adverse findings that may be directly associated with the desired genotype. Describe criteria for monitoring the well-being of animals and criteria for terminating/modifying the procedure if adverse effects are observed. Describe how the signs listed above can be ameliorated or alleviated. Please provide scientific justification if signs are not to be alleviated or ameliorated.

Even if pain or distress is not anticipated, the protocol must contain a contingency plan for dealing with unexpected situations that may arise. The plan should include detailed written criteria for the humane endpoints that will be used to determine when animals will be removed from the study, treated, or euthanized. The Animal Welfare Regulations and Public Health Service Policy state that animals that would otherwise suffer severe or chronic pain and distress that cannot be relieved should be painlessly euthanized at the end of the procedure, or if appropriate, during the procedure.

It is important to note that if any unanticipated adverse effects not described in the protocol occur during the course of the study, a complete description of these unanticipated findings and the steps taken to alleviate them must be submitted to the IACUC as an amendment to the protocol.

**14. Method of euthanasia:**

Even if your study does not involve euthanizing the animals, you must show a method that would be used in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Recommended Methods

The 2000 Report of the AVMA Panel on Euthanasia categorizes methods of euthanasia (see below). A copy of the AVMA report can be accessed at
http://www.avma.org/issues/animal_welfare/euthanasia.pdf

**Acceptable**

o Barbiturates (most species)
o Carbon dioxide ($CO_2$) – compressed gas only (most species)
o Inhalant anesthetics (most species)
o Tricane methane sulfate (TMS, MS222) (fish, amphibians)
o Benzocaine hydrochloride (fish, amphibians)
o Ether and carbon monoxide are acceptable for many species, but relatively dangerous to personnel.
o Captive penetrating bolt (horse, ruminant, swine)

**Conditionally Acceptable (Requires Scientific Justification and IACUC approval)**

o Cervical dislocation (birds, small rodents and rabbits)
o Decapitation (birds, rodents, some other species)
o Pithing (some ectotherms)
o Various pharmacological and physical methods

**Unacceptable**
o Chloral hydrate, chloroform and cyanide
o Decompression
o Neuromuscular blockers
o Various pharmacological and physical methods
o Dry ice – generated CO2.

**15. Disposition of animals**
Please describe what will happen to the animals at the end of the study. If they will not be euthanized, then please include this statement. Specifically describe study end points and indicate the time point, if any, when animals will be euthanized Please note: The Raptor Center can only accept healthy rodents that have not been infected with pathogens or treated with drugs, anesthetics, or toxins.

**16. Project Roster**
The protocol form provides substantial information on the requirements associated with training, occupational health, and documenting all individuals associated with the animal protocol. Please follow each of the steps identified, indicating for each person: name, employee number OR e-mail address, and training and experience relevant to the procedure described in this protocol, for example; classes attended, hands on training, years of experience, or surgical experience. Only those individuals that will be working with live vertebrate animals need to be included. If individuals are working with animal tissue only or after the animal has been terminated, then they need not be included. Animal care and vet staff associated with the vivarium do not need to be individually listed. Failure to provide this information may significantly delay approval of your protocol.