ANIMAL USE APPLICATION FORM SUBMISSION GUIDELINES

The University of Central Florida Institutional Animal Care and Use Committee (IACUC) meets on a bi-monthly basis to review animal use protocols. The bi-monthly meetings are held the following months. January, March, May, July, September and November.

The IACUC reviews protocol submissions either at a convened meeting of a quorum of members known as full committee review (FCR) or through the use of designated member review (DMR).

- **Required Items For IACUC Review:**
- **All Items must be answered. If not applicable, please state "NA".**
- **Forms must be type written**
- **Form must have PI and Department Chair signatures**
- **A Word version of the form must be submitted by E-mail to** IACUC@UCF.edu
- **Signed Hard Copy Forms should be submitted to:**
  Cristina Caamaño, Associate Director Office of Animal Welfare
  University of Central Florida
  12201 Research Parkway, Suite 501,
  Orlando, FL 32826-3246
  (OR)
  If you are located on the Lake Nona campus, forms may also be dropped off in the IACUC mailbox of the Burnett Building at Lake Nona.
- **CITI training must be completed by all personnel listed on the protocol prior to submitting the form to our office.** For more information on CITI, please click on the training tab on our website
  http://www.research.ucf.edu/Research/OfficeOfAnimalWelfare.html
- **Type of Project** (Note: Category C, D, and E Projects Require Veterinary Signature and Category E projects require Full Committee Review
- **Investigators should plan for at least 3 to 4 weeks between the time a protocol is submitted to the IACUC and final approval is granted. If your project is type "E" you must submit your protocol at least two months in advance as this protocol must be reviewed at a convened meeting. Please submit your protocol at least two months in advance if your project contains multiple survival surgeries as this *may* also require full committee review.**

Note: For any questions about this form, please contact the Office of Animal Welfare at: Tel: 407-882-1164; Fax: 407-823-3299; email: IACUC@ucf.edu

The IACUC Form is available in both PDF and DOC format. Please regularly check the website to ensure that you have the most updated version of this application form.

University of Central Florida

FOR UCF/IACUC OFFICIAL USE ONLY:

IACUC Number: _____ **1st year approval:** _____ **DMR / FCR**

Receipt Date: _____ **2nd year approval:** _____ **DMR / FCR**

Submission Type: **3rd year approval:** _____ **DMR / FCR**

____ New application ____ Revised application ____ Renewal with Changes

IACUC Action: ____ **Approved on** _____

ANIMAL USE APPLICATION FORM

INSTITUTIONAL ANIMAL CARE & USE COMMITTEE
University of Central Florida
Office of Animal Welfare
12201 Research Parkway, Suite 501
Orlando, FL 32826-3246

IACUC Contact: Mrs. Cristina Caamaño
Tel: 407-882-1164; Fax: 407-823-3299; email: IACUC@ucf.edu

Check as applicable:
New application ___ Revised Application ___ Renewal application including changes ____
Type of Project:
Category A __ Category B __ (Veterinarian signature **is not** required)
Category C __ Category D __ (Veterinarian signature **is** required)
Category E __ (Veterinarian signature and Full Committee Review are required)
(For a description of the above categories, please refer to section C of this form)

Responsible Faculty Name: _____
Campus Address: _____
Department: _____
PI Phone: _____ **Fax:** _____ **E-mail:** _____
Name of Dept. Chair: _____
Co-PI (s): _____
Technicians Involved: _____
Students Involved: _____
Others Involved – please specify (e.g. Animal Care Team): _____
_____

Section A.

1. TITLE OF PROJECT*:* The title should be identical to the one submitted to the funding agency. If more than one title applies, list them all here, and indicate which funding agency applies to which title.

2. SOURCE OF FUNDING: If the proposal is sent to more than one agency and uses more than one title, please explain. Once a project is awarded and funds received, the other proposals usually become void under this protocol approval. When federally funded, each animal project must have its own Animal Use Approval Form and number. Any exceptions to this should be justified here and approved by the IACUC.

3. IS YOUR PROPOSAL SUBJECT TO INDEPENDENT PEER (MERIT) REVIEW? Type YES or NO. If YES, please specify by whom, i.e. NIH study section. If NO, please explain why there has been no merit review.

4. DESCRIPTION OF ANIMAL PROJECT IN NONTECHNICAL TERMS: This should include a statement of your experimental hypothesis (or teaching objectives) and be written in lay terms so it can be understood by the general public. **Include in your description what possible contributions your work might make to the broad disciplines of human/animal well-being or the expansion of human knowledge.**

- To make your explanation understandable to the lay public it is essential to use simple, non-technical language (high school level).
- Failing to adequately describe a project in non-technical terms, failing to state the hypothesis, are several common reasons why this approval process is delayed. Please do not submit your proposal abstract for this project description - most are too technical for the public to understand.

5. PROPOSED STARTING DATE*:* If this is an ongoing project, list the original starting date of this project.

New Project: Month: _____ Day _____ Year_____

Ongoing project/original dates: Month: _____ Day _____ Year_____

6. NUMBER OF YEARS PROJECT IS PLANNED TO CONTINUE and/or PROGRESS REPORT OF CONTINUING PROJECT (if applicable)*:* If this is a new project, how long will it continue? Please note: Protocols are approved for a maximum of three years. If this project has been

ongoing for several years, or **if you are renewing your old Approval Form**, please provide a brief explanation or progress report at this time. The Committee is especially concerned about how many animals have already been used and how many more are being requested at this time.

7. LIST SPECIES OF ANIMAL(S) TO BE USED, THEIR CLASSIFICATION STATUS and LIST THE TOTAL NUMBER OF ANIMALS TO BE USED FOR THIS PROJECT: Common names for the exotic species are helpful. If 5 animals are Type D and 25 animals are Type C, then list them that way. The total number must be justified in Item 9. Please use the following table format. *If you are not familiar with the categories, please review their definitions in Section B of this Form.*

SPECIES	**CATEGORY (A, B, C, D, E)**	**TOTAL # REQUESTED FOR 3 YEARS**

8. JUSTIFICATION FOR USING THIS PARTICULAR SPECIES*:* Why are you requesting to use this species? Common statements have included: animal size; compatibility with previous studies; known susceptibility; existing knowledge database; and the information is intended for use in this species.

9. JUSTIFICATION OF YOUR ANIMAL NUMBERS: It is necessary that sufficient detail be provided so that the number of animals requested in Item 7 can be understood by the IACUC. It is helpful to explain animal groupings, any replications and anticipated wastage or training usage. It is important that actual numbers in experimental groups be justified using the best information available to you at this time. Please refer to the *"IACUC Policy on Counting Vertebrate Animals"* for guidance. It is very important to list the time intervals between the beginning of the animal's role to the end of the experiment. If you are updating a project that has been ongoing, please indicate how many animals have already been used, and how many more are needed to finish the project.

This number should match the number you listed in item 7. For multiple year projects, you can list the animals expected to be used each year, but it is the total number of animals for the project that is important for approval.

Sample Size. State the proposed sample size and estimate (or explain) the statistical power related to testing your hypothesis.

A table is usually a good way to present this information:

	20 min post inj.	40 min Post inj.	60 min Post inj.
Steroid Group	n= 4	n= 4	n= 4
Non-steroid Group	n= 4	n= 4	n= 4
TOTAL	**8**	**8**	**8 = 24 + 5 = 29**

If additional animals are needed for training or for replacements, please make an additional entry here as well. *For example, 5 additional animals will be needed to refine this technique or to train personnel before we can actually start the study.*

9-1. Approximately, how many animals will be needed for this project? Please indicate Male / Female.

9-2. How many experimental groups, replications, trials, etc. are required?

9-3. How did you determine that the sample size, number of groups, replications, trials, etc. are appropriate, as they relate to the number of animals requested?

Or, if your animals are being used for instructional purpose please supply the following additional details:

(a) Average class size

(b) Teacher to student ratio

(c) If off-campus, give location and details of veterinary supervision, if needed.

(d) If you use a syllabus or provide handouts regarding the use of animals, please include them as an appendix to this Animal Use Approval Form

_____ Appendices are included as follows: _____ (1); _____more than 1

10. IS ANIMAL BREEDING BEING DONE FOR THIS PROJECT? Type YES or NO. If YES, please complete Appendix 1 on this form. *Refer to section B for guidelines.*

11. ENVIRONMENTAL HEALTH & SAFETY QUESTIONS:

11-1. WILL ANY HAZARDOUS AGENTS BE USED WHILE PERFORMING THIS RESEARCH? Answer YES or NO. If the answer is YES, please list the agent. *Refer to section D. at the end of this document for definitions of each category.*

- **Pathogens/Tissues**

- **Recombinant/Synthetic Nucleic Acid**

- **Blood borne Pathogens**

- **Chemicals/Toxins**

- **Animal Care Drugs**

- **Radiological Agents**

- **Others**

11-2. INDICATE IF ANY HAZARDOUS PROCESSES WILL BE INVOLVED WHILE PERFORMING THIS RESEARCH.

- **X-ray/Laser or Others**

11-3. PROTOCOLS INVOLVING HAZARDOUS AGENTS AND/OR PROCESSES REQUIRE REVIEW AND APPROVAL FROM THE UCF DEPARTMENT OF ENVIRONMENTAL HEALTH & SAFETY PRIOR TO PROJECT IMPLEMENTATION.

The EH&S Hazardous Agents and Process Identification (HAPI) form is located on the EH&S website: http://www.ehs.ucf.edu/biosafety/HAPI-Form.pdf

Check as applicable:

_____ Completion of the form is not applicable.
_____ Form has not been submitted, but will be submitted before project implementation.
_____ Form has been completed and it is pending review and approval by The UCF's EH&S Office.
_____ Form was processed. The EH&S HAPI protocol number is: _____

Please refer to section D. at the end of this document for additional requirements concerning special hazards on your research.

12. WHERE WILL YOU GET YOUR ANIMALS? Please indicate who will order and/or purchase these animals for you. If you know the source of these animals, please list that source here.

NOTE: Approval must be obtained from the facility managers at least two weeks prior to placing animal orders. Only animals from approved vendors are allowed directly into the animal facilities.

13. WHERE WILL YOUR ANIMALS BE HOUSED? Please indicate the building or housing unit where you will keep these animals.

14. REMOVING ANIMALS FROM THE ANIMAL FACILITY

14-1. WILL YOU REMOVE LIVE ANIMALS FROM THE ANIMAL FACILITIES? Please indicate YES or NO and specify any non-centralized housing and/or procedural area on the table provided.

NOTE: Live animals cannot be kept in the laboratory/outside the vivarium overnight.

Location of non-centralized housing and/or procedural area	Purpose

14-2. DESCRIBE HOW THE ANIMALS WILL BE TRANSPORTED.

15. WHAT WILL HAPPEN TO YOUR ANIMALS AT THE END OF THIS PROJECT? Select one of the following options and explain as shown below. Please answer for all the species listed in item 7.

Transfer to other projects (explain):

Adoption or resale (explain):

Euthanized Explain the method to be used for euthanasia of all animals, including live birth pups. If you are using a drug(s), you must indicate the name, dose and route of the drug. If the method you use is not approved by the AVMA, you need to justify it and have it approved by UCF's IACUC. If you are unsure about your method of euthanasia, please consult the UCF Attending Veterinarian.

Example: *"When possible, animals will be euthanized in their home cage with CO_2 at a rate of 10-30% volume displacement per minute. When respiration and heartbeat appears to have stopped, cervical dislocation will be performed to ensure death". (Other methods include exsanguination, thoracotomy).*

Cervical dislocation and decapitation of animals requires prior sedation. If you cannot sedate the animals prior to cervical dislocation or decapitation, you must give good reasons to justify the withholding of sedatives. Please put that justification statement here in Item 15. Also, if you cannot sedate animals prior to cervical dislocation or decapitation, you are required to describe the training or experience acquired for the person using this method – please describe that training or experience.

16. PROPOSED ACTIVITIES OR SIGNIFICANT CHANGES IN ONGOING ACTIVITIES AS STATED IN RESEARCH PROTOCOLS MUST MEET THE FOLLOWING FEDERAL REQUIREMENT: The following requirement is only for Category Type C, D or E procedures.

*"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 minimal written narrative description of the databases searched or other sources consulted, the date of the search and the years covered by the search, and the key words and/or search strategy used by the principal investigator. Examples of sources: biological abstracts, index medicus. Medline, CRIS, Animal Welfare Information Center (AWIC**)."*** **If your research fits in the above C, D or E category, you must make a narrative statement here in Item 16**.

A sample narrative statement could be as follows:

> *"I have considered alternatives to the use of (identify the painful procedure) and have found none that are available. The database(s) searched included a (list the date of the search) search of (list databases) for the years (list the range of years searched) of the words (list the key words searched)".*

Or you can make a similar narrative statement regarding your consideration of alternatives.

Electronic Databases Searched or Sources Consulted	**Years Covered By the Search**	**Date (MM/DD/YY) of the most recent search performed**	**Keywords used for the search (e.g. "procedure + species + refinement + alternative")**

17. DESCRIPTION OF ALL ANIMAL PROCEDURES TO BE USED IN THIS STUDY:

The IACUC needs to know what procedures will be done to these animals. If you are performing injections, inoculations or blood withdrawals, describe the dosages, sites, volumes, routes, and schedules involved; radiation (dosage, isotope and schedule); restraining procedures if longer than several hours (e.g., restraint chairs, collars, vests, harnesses, slings, chutes etc.); and any other procedures (excluding surgery) must be described here. If these procedures are to be performed outside the vivarium or animal holding area (i.e. lab) please refer to Item 14. Description of surgical procedures should be given in Item 27 and/or Item 28.

Please use bullets and a potential timeline of the procedures so that the overall procedure is easily understood. You may also use section headers, for example BLOOD DRAWS, then underneath describe the four different ways you potentially would draw blood.

18. WHAT ARE THE EFFECTS OR SYMPTOMS YOU EXPECT TO SEE FROM THESE ANIMALS?

Will you expect the animals to experience pain or discomfort? Indicate the nature of the pain and distress, how it will be recognized, and what measures you will take to either end the distress or provide relief.

If you expect any animals to develop any clinical conditions or abnormalities (including changing tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, changes in clinical signs, or signs of toxicity, weight loss, etc.) please provide details here.

If supplemental supportive care can be provided, specify the type of care. Examples include: placing the cage on a heated surface, placing moistened food pellets on the cage floor, adding dietgel and/or hydrogel, administering saline or lactated ringers subcutaneously.

If you are providing analgesics supply their generic names, route of administration, doses, and dosing intervals. In answering this section be accurate regarding drugs and doses. A consultation with a veterinarian may be advisable if you are unfamiliar with the species or the extent of the pain.

If death is used as an end point, explain why they cannot be euthanized.

If you do not expect to see any symptoms, or if symptoms occur that you did not expect, please make a statement that describes how often you will observe your animals, and if symptoms do occur, the veterinary staff which will be contacted.

19. WILL ANIMALS BE SUBJECTED TO ANY NON-STANDARD HOUSING OR CARE AS PART OF THIS STUDY? Type YES or NO. If "YES" explain how housing & care would differ and provide justification. Examples include: less frequent bedding changes in pheromone study; hyperbaric chamber used as primary enclosure; specially made cages for behavioral study, request for different diet and dosing water bottles.

20. WILL ANIMALS BE SUBJECTED TO EXCESSIVE RESTRAINT WITHOUT THE USE OF ANESTHESIA? Type YES or NO. The amount of restraint usually performed with experimental animals is minimal (minutes rather than hours). If "YES" explain and provide justification.

21. WILL ANIMALS EXPERIENCE ANY ELECTRICAL SHOCK? Type YES or NO. If animals must receive electric shocks as part of your study, provide details as to intensity, length and frequency of stimulus. It is important to also explain the circumstances of the shock and if the animal can get away from it. Detailed justification is required.

22. WILL FREUND'S COMPLETE ADJUVANT BE USED? Type YES or NO. If FCA must be used, you must explain why you have chosen not to use other less irritating adjuvants, like RIBI or TiterMax. Provide details of the concentration of FCA in the injection, total volume injected, routes, sites and number of injections).

23. **WILL ANIMALS UNDERGO ANY FOOD OR WATER RESTRICTION IN EXCESS OF 24 HOURS? THIS INCLUDES FASTING FOR SURGERY.** Type YES or NO. If you are withholding food and/or water from animals **in excess of 24 hours,** explain why this is necessary and how you will monitor the animals to ensure that they are not excessively stressed by the protocol. The daily monitoring of body weights is usually required for food and/or water restrictions in excess of 24 hours.

24. WILL ANIMALS BE SEDATED OR ANESTHETIZED FOR RESTRAINT OR SURGERY? Type YES or NO. If you are sedating or anesthetizing your animals, provide details of the drugs (generic names), dose, and route, along with details of how you will monitor the animal's level of sedation or anesthesia. These anesthesia and monitoring details are needed for both survival and non-survival surgical procedures.

25. WILL PARALYZING AGENTS (MUSCLE RELAXANTS, i.e.: PANCURONIUM) BE USED TO RESTRAIN YOUR ANIMALS? Type YES or NO. If your protocol calls for the use of muscle relaxants that produce paralysis of your animals, explain why this is necessary and how you will determine that an animal is not without anesthesia, awake, and fully able to experience pain. In situations where there is no surgery or painful procedure involved and paralysis has to be used in conscious animals, full justification will still be required as well as details of how the animal's comfort needs will be met. Investigators unfamiliar with the use of muscle relaxants in animal species should seek a veterinary consultation as there are very marked species differences that could void a study if unappreciated.

26. WILL YOU BE USING AN INHALANT ANESTHETIC? Type YES or NO. Specify the anesthetic, provide a description of how the anesthetic will be administered including the equipment to be used, and state how the animal will be monitored. Anesthetic waste gases are classified as environmental hazards. These gases must be used in a fume hood or have a gas scavenging system in place. The use of diethyl ether, as an anesthetic agent, is prohibited because of its flammability.

Sample Narrative: *"YES. Isoflurane will be administered through a certified anesthesia unit fitted with an activated charcoal canister (used to absorb waste anesthesia gases). The animal will be placed in a chamber for anesthesia induction at a rate of 2-4% isoflurane with an oxygen flow rate of 0.5-1L/min. When the animal is observed to be unconscious, it will be removed from the chamber and moved to a nosecone to continue flow of anesthesia. Plane of anesthesia will be confirmed by toe pinch and/or palpebral reflex. Water soluble ointment will be applied to eyes to prevent corneal desiccation. Rate of anesthesia may be adjusted between 1-3% for the duration of the procedure/s."*

27. WILL YOU BE PERFORMING NON-SURVIVAL SURGERY? Type YES or NO. If you are performing surgery from which the animal does not recover consciousness, please explain the surgical procedures in detail. Include the surgical approach and organs involved. It is also necessary to describe the monitoring and supportive care provided during surgery. Describe how long the animals will be anesthetized during this procedure and when euthanasia occurs. In addition, the means of maintaining and monitoring body temperature, fluid balance, heart and respiratory rate should also be described. Particular emphasis should be placed on how you will ensure the animal does not recover consciousness and how euthanasia is performed.

28. WILL YOU BE PERFORMING SURVIVAL SURGERY? Type YES or NO. Please provide a description of the surgical procedure, the immediate recovery care, and post-operative care. Indicate the use of aseptic techniques; this may include the use of sterile gloves, sterile instruments, disinfection of the surgical area, etc. Include the surgical approach, organs involved, implants and method of skin closure. The use of silk to close the skin is not acceptable. Please identify a nonwicking material to use to close the skin. It is also necessary to describe the monitoring of body temperature, fluid balance, and heart and respiratory rate. Indicate what arrangements will be made for providing post-operative care after normal duty hours, weekends, and holidays.

29. WILL MORE THAN ONE SURGICAL PROCEDURE BE PERFORMED (Multiple Survival Surgery)? Type YES or NO. If more than one major survival surgical procedure is to be performed on an individual animal, explain the procedures and their sequencing. Scientific justification is required if an animal is to be used for more than one major operative procedure from which it is allowed to recover. Major surgery is defined as penetration and exposure of a body cavity and/or any procedure that has the

potential to produce permanent impairment of physical or physiological functions. All authorities are quite clear - economic considerations are not considered an adequate reason for multiple surgeries on an individual animal).

30. LOCATION OF ANIMAL SURGERY: Please indicate YES or NO if animal surgery is to be performed anywhere other than in the animal facilities. Please provide the name and building number along with the room number.

31. IS THIS A TYPE "E" STUDY? Type YES or NO. If your study is a Type E (no relief of pain is permitted), the University is required to submit a justification for this study to the federal agencies. Include in your justification statement below, an explanation of why the use of analgesics or euthanasia is not permitted. It is also required that investigators consult with appropriate veterinarians before submitting their application. Make sure you have the signature of a DVM that indicates this consultation has occurred.

32. PLEASE LIST ALL PERSONNEL CONTACTING ANIMALS: Please list all persons involved with this project that will be contacting animals. Persons listed below will be contacted with information regarding this program.
It is highly recommended that persons listed below be immunized for tetanus if there is a risk of exposure.

Name	Department	Phone
1.		
2.		
3.		
4.		
5.		

33. PLEASE LIST TRAINING OF ALL PERSONNEL LISTED ABOVE: It is required that personnel be properly trained in the animal procedures you have listed in this project. This includes animal handling, restraint and the euthanasia methods listed. Indicate their training and experience below, who has trained them and where they were trained. Also list how long they have been doing these procedures. You may also list any animal workshops attended. Please place a checkmark indicating completion of CITI training, as CITI online training must be completed prior to protocol review by the IACUC.

Name (Academic Degrees/Certifications)	Training, Relevant Experience or Years Performing Procedures	CITI Training Completed

34. WILL PRIVATELY OWNED ANIMALS BE USED? Type YES or NO. If your experimental subjects are not procured or owned by the University, but are privately owned, then you are required to explain how the animals are obtained/recruited. If the animals have a need for veterinary services, who will be providing those services?

35. NAME and SIGNATURE OF VETERINARIAN CONTACTED FOR CONSULTING ON PAINFUL TECHNIQUES OR PROCEDURES (Categories C, D and E only*:*

On Types A and B studies, this name is not required. **This requirement is only for those projects that are type C, D or E**. For Type C, D or E studies, you must list a name for the person providing this service.

_____ _____

Name of Veterinarian (please print) **Signature of Veterinarian** **Date**

36. FACULTY ASSURANCE STATEMENT: *"The information contained in this application for animal use approval is accurate to the best of my knowledge and the project does not unnecessarily duplicate previously performed experimental work. Appropriate space and funding have been arranged in the event that the project is approved. All personnel listed recognize their responsibility in complying with University policies governing the care and use of animals and have received or will receive appropriate training specific to the procedures outlined in this project." (Required)*

PI assures the University of Central Florida and its IACUC that all required documentation (Such as the FL State Exemption Letter and DEA Registration) will be obtained to carry out the proposed research prior to project implementation.

_____ _____

Name of Responsible Faculty (please print) **and** **Name of Department Chair** (please print)

_____ _____ _____ _____

Signature of Responsible Faculty **Date** **and** **Signature of Department Chair** **Date**

Appendix 1

Breeding Colony Information

1. **HOW WILL YOU MAINTAIN THE GENETIC INTEGRITY OF THE BREEDING COLONY?** (If your animals are inbred or outbred, appropriate nomenclature must be

used and the breeding pairs and generations recorded (usually on cage cards and record books).

2. **PLEASE DESCRIBE THE FOLLOWING DETAILS AS BEST YOU CAN:**

   A. **1:2 Male to female ratios?** *Note: At least 1 female should be removed prior to either female giving birth to prevent multiple births in a cage.*

   B. **Any chemicals or methods to synchronize estrus?**

   C. **Caging type (ventilated, filtered or conventional)?**

   D. **When are pregnant females removed from male?** *Note: At least 1 female should be removed prior to either female giving birth to prevent multiple births in a cage.*

   E. **When to cull old breeders?**

   F. **How many litters permitted per dam?**

   G. **How many breeding pairs at any one time during the year?**

3. **WHO IS THE ONE PERSON (lab manager, post-doc, technician, student, etc.) RESPONSIBLE FOR THE DETAILS OF THIS BREEDING PROGRAM?** If this is not the Responsible Faculty (PI) member listed above, please identify that person here (phone and & email).

4. **WHY DO YOU WANT TO MAINTAIN THIS COLONY AT UCF RATHER THAN PURCHASE ANIMALS FROM AN APPROVED VENDOR?**

5. **RODENT BREEDING COLONIES ALMOST ALWAYS PRODUCE MORE ANIMALS THAN ARE NEEDED. PLEASE DESCRIBE HOW YOU INTEND TO CULL THOSE OFFSPRING NOT ACCEPTABLE FOR YOUR RESEARCH AND THOSE OFFSPRING THAT ARE NO LONGER USEFUL. WHEN WILL THESE DECISIONS BE MADE (age, weight, and test results)?**

6. **ANY GENOTYPING OR OTHER PROCEDURE DONE ON ANIMALS IN THIS BREEDING COLONY MUST BE DESCRIBED HERE. ESPECIALLY ANY INVASIVE PROCEDURES.**

7. **WILL YOU BE AGING ANY OF THE ANIMALS FROM YOUR BREEDING COLONY? IF SO HOW, MANY WILL BE AGED? HOW OLD WILL THEY BE ALLOWED TO GROW? IS THIS A REASONABLE EXPECTATION FOR THIS STRAIN OF RODENT?** *PLEASE EXPLAIN.*

End of Section A.

Section B.

FOR REFERENCE ONLY

UCF Guidelines for Rodent Colony Breeding & Management

Requirement: If the breeding animals come under considerations described in your IACUC protocol, then all activities of breeding (below) must be reviewed and approved. The following items are presented as Guidelines for you to consider and address if they apply to your research.

The goal of managing any mouse colony at UCF should be to maintain adequate numbers of animals in as little shelf space as possible, while adhering to the university's policies regarding health and well-being of the mice, and minimizing labor costs. What constitutes "adequate numbers" will, of course, depend on a number of research factors, including:

- experimental needs,
- breeding characteristics of a given strain,
- genotypes and phenotypes (morbidity/mortality) of individual mice,
- limits of approved animal use protocol.

These guidelines present only the minimum effort and cage space needed to keep a normal strain of mice "on the shelf". If more animals are needed, more cage and shelf space result. However, the principles of mouse numbering, culling, and replenishment can be utilized on any scale to minimize cage space.

Different strains of mice vary in fecundity, and certain mutant strains can be difficult to breed, due to a variety of factors such as small litter sizes, low fertility, poor mothering instincts, high rates of cannibalism of newborns, and higher morbidity or mortality resulting from the genetic mutation/alteration. When acquiring a new genetically modified strain, therefore, it is always a good idea to consult with someone who has direct experience with maintaining that strain.

Cages required to simply maintain a strain: Simple strain maintenance generally requires no more than 2-3 mating cages and 3-4 additional cages to hold weaned pups that will be used to replace old breeders. To predict the cages needed to produce mice for experiments, figure about one litter per month from young breeders, and about 6-8 pups/litter, until experience proves otherwise. Maintaining these production levels requires consistent replacement of older breeders.

Breeding: House breeders in pairs of one male and one or two females. Gestation lasts 19-21 days. Check cages at least twice each week to flag pregnant females and record approximate birth dates. Pups are usually weaned at (removed from breeding cage and separated by sex) at 3 weeks of age. Identification can be accomplished prior to weaning by any of several methods (see below). Tissue for genotyping is usually collected at the same time that ID numbers are applied.

Weaning: The male and female pups are usually weaned (removed from breeding cage and separated by sex) at 3 weeks of age and moved to separate holding cages, with no more than 5 mice per cage. Females of any age or breeding status can be housed together. To minimize fighting, weaned males should be group-housed only with their littermates, and only until they are exposed to females. Males from different litters can be grouped together only if they are no more than 4-5 days apart in age. Even littermates may have to be separated as they age, to prevent fighting. Males that have been used as breeders must ALWAYS be housed singly, because they will kill each other.

Replacement of breeders: Replace a breeder pair if:

- they have not produced a litter in two months,
- they are producing small litters (1-3 pups per litter),
- they are killing their pups.

New breeder pairs should be 8-12 weeks of age. Although both males and females can breed up to 8 months of age and beyond, if new breeders are available there is little point in continuing to use breeders older than about 6 months. Also, a mouse that has not been allowed to breed prior to about 3-4 months of age may never breed successfully. As breeders age, both litter size and the frequency of litters decrease. It may be wise to wait for new breeders to produce a litter, before euthanizing old breeders, if the strain presents any unusual breeding problems.

Cull older progeny: Euthanize older offspring as new litters are weaned. It is easy to neglect weaned pups, since they are not breeding. Periodic evaluation of all cages is essential to minimizing cage usage. If progeny are more than 3 months old, and you have new litters to replace them, and you don't need

them for experiments, then take them off the shelf. The only reason to keep more than one or two weaned litters for a strain that is not being used in experiments is if that strain exhibits unusual morbidity or mortality, or is otherwise difficult to breed.

Pup identification: Weaned pups from different litters may be housed together if they are uniquely identified (or if they don't need to be identified). This helps to minimize the number of holding cages. (See above, however, for caveats about males.) The following table lists the common methods of pup ID, with pros and cons of each.

Method	pros	cons
ear punching/notching	Simple, inexpensive, easy to read	Sometimes ambiguous, subject to tearing and healing, limited numbers
metal ear tags	Unique numbering, relatively inexpensive	Loss of tags, infections, hard to read
toe clipping	Simple, inexpensive, permanent	Less humane, must be done at an early age
tattooing	Relatively permanent, easy to read, may be done on newborns	More difficult and time-consuming, may fade with time if done improperly
microchips	Permanent, virtually unlimited numbers, can provide physiologic data	High cost per mouse, difficult to apply, requires expensive reader

End of Section B.

Section C.

FOR REFERENCE ONLY

ANIMAL USE CLASSIFICATION (CATEGORIES A, B, C, D, E)

In the ***Animal Use Approval Forms*** you are asked to classify the project according to the level of perceived pain / stress / distress.

TYPE A:

STUDIES ON NON-LIVING VERTEBRATE ANIMAL MATERIAL, NON-INVASIVE OBSERVATIONS OF WILDLIFE, AND/OR WHERE THERE IS NO CONTACT WITH ANIMALS

These include vertebrate animal tissues obtained at necropsy, slaughterhouse, or meat markets (grocery stores), observational studies on wildlife and other animals that do not involve physical restraint or handling are included. Also included in this category are projects that use commercial or other USDA registered animal facilities to produce animal products, like commercial antibody companies.

TYPE B:

STUDIES ON LIVE, VERTEBRATE ANIMALS CAUSING NO MORE THAN MINIMAL PAIN OR DISTRESS

Examples include: routine examinations; blood sampling; injection of non-toxic materials; approved methods of euthanasia that induce rapid unconsciousness; short periods (up to 24 hours) of withholding food and water. Acceptable levels of minimal pain and discomfort in this category would be those procedures that are normally done on animals given routine physical examinations at veterinary clinics. Animals that are euthanized and then have tissues/organs removed are included in Type B. Animals that are anesthetized and then have tissues/organs removed before euthanasia are in Type C.

TYPE C:

STUDIES INVOLVING MORE THAN MINIMAL (MILD) PAIN OR DISTRESS USUALLY OF SHORT DURATION

Examples include invasive studies on COMPLETELY anesthetized animals that may or may not regain consciousness. Survival surgical procedures that may result in minor post surgical discomfort. Also included are studies using noxious stimuli from which escape is possible; some tumor or device implants; the use of Freund's complete adjuvant; and domestic animal production methods (following accepted veterinary practices), i.e. tail docking, neutering, dehorning, debeaking, etc.

***Comment*:** Animals are not expected to show prolonged (days) clinical symptoms, other than some mild discomfort, during or after Type C procedures. Terminal invasive procedures done on anesthetized animals before they are euthanized are included as Type C.

TYPE D:

STUDIES INVOLVING MODERATE TO SEVERE PAIN OR DISTRESS, BUT THIS PAIN OR DISTRESS IS ALLEVIATED OR OTHERWISE CONTROLLED BY DRUGS

Examples include major surgery under general anesthesia that results in significant post-operative discomfort, prolonged periods (several hours or more) of uncooperative physical restraint; deprivation of the animals' environmental necessities, such as maternal deprivation; aggression; and predator-prey interactions. Also included are studies in which diseases or

toxicities are induced and the animals are expected to become sick or abnormal. Animals in Type D studies may experience pain, but the necessary treatments to alleviate the symptoms are available and provided, or the animals are euthanized. Involvement of trained technicians, scientists, and veterinarians is critical if this pain is to be minimized or avoided. Adherence to acceptable veterinary practices is mandatory and will vary depending on the project, i.e.
Post-op analgesia, fluid therapy or intensive nursing care.

***Comment*:** Animals are expected to show clinical symptoms of pain or distress as a result of the research objectives, but these symptoms are treated or otherwise alleviated with the use of drugs or intensive care.

TYPE E:

STUDIES INVOLVING SIGNIFICANT PAIN OR DISTRESS WITHOUT THE BENEFIT OF PAIN-RELIEVING DRUGS.

Examples include: application of noxious stimuli from which escape is impossible; the use of muscle relaxants in surgery without concurrent use of anesthetics, induction of aggressive behavior leading to self-mutilation or fighting where death is the end-point. Also included are studies in which death is the end-point, i.e. diseases are induced and infected animals are permitted to succumb rather than be treated.

Comment: Animals are expected to show clinical symptoms of pain or distress as a result of the research objectives, but these symptoms cannot be treated or otherwise alleviated with the use of drugs or intensive care because doing so would interfere with the research objectives. Type E studies place an explicit responsibility on investigators to explore alternative designs to ensure that these methods have to be used.

IMPORTANT: The reasons for using these procedures must be explained in a statement by you, the Principle Investigator, justifying their use. This statement is requested in the last item of the ***Animal Use Approval Form***. This statement is required by federal law. The IACUC submits this statement in annual reports submitted to the government.

End of Section C.

Section D.

SPECIAL HAZARDS

Should the project involve the use of any of the following special hazards, completion of the Hazardous Agent or Process Identification form is required and approval by the

UCF's Environmental Health and Safety Office must be received prior to the project implementation.

Examples of hazardous agents

- **Pathogens/Tissues:** Agents, used experimentally in animal use protocols, of biological origin that are potentially pathogenic, or may otherwise cause disease processes in animals or humans including risk group 1,2,3 and novel infectious agents.

- **Recombinant/Synthetic Nucleic Acid:** Recombinant and synthetic nucleic acids as defined by the NIH Guidelines (including exempt experiments, infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus), used experimentally in animal use protocols.

- **Blood borne Pathogens:** Including human tumor cells, human and non-human primate blood and body fluids, or cells lines immortalized with a virus used experimentally in animal use protocols.

- **Chemicals/Toxins:** Agents of chemical or biological origin, used experimentally in animal use protocols that are potentially toxic, carcinogenic, mutagenic or corrosive to humans or animals tissues, or are considered to be flammable or reactive hazards.

- **Animal Care Drugs:** Agents used in the course of animal care: includes controlled substances, prescription drugs, and non-prescription drugs that may pose a health or physical hazard to animal care workers.

- **Radiological Agents:** Agents, used experimentally in animal use protocols that can potentially cause damage to human or animal tissues by emitting alpha, beta, or gamma radiation.

Examples of hazardous processes

- **X-ray or Laser:** Equipment used experimentally in animal use protocols that can potentially cause damage to human or animal tissues by emitting ionizing or non-ionizing radiation.

The Hazardous Agent or Process Identification (HAPI) form can be found at:

http://www.ehs.ucf.edu/biosafety/HAPI-Form.pdf

End of Section D.