

**Institutional Animal Care and Use Committee
Policy and Procedure Manual**

Institutional Animal Care & Use Committee,
Office of Research & Commercialization, University of Central Florida

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I. University Policy Regarding Use of Animals in Research, Teaching, or Testing

I.1. The University of Central Florida (University) affirms that respect for all forms of life is an inherent characteristic of biological and medical scientists who conduct research involving animals, that the respectful treatment, care and use of animals involved in research is an ethical and scientific necessity, and that the use of animals in research and teaching contributes to the advancement of knowledge and the acquisition of understanding.

I.2. The University has established and provides resources for an Animal Care and Use Program that is managed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Guide), the *Animal Welfare Regulations Title 9 Code of Federal Regulations Subchapter A, "Animal Welfare", Parts 1-3* (AWR), and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy). . Studies of wild animals in natural settings are conducted in accordance with the *Acceptable Field Methods in Mammalogy, Guidelines for the Capture, Handling, and Care of Mammals*, the *Guidelines for Use of Fishes in Field Research*, the *Guidelines for Use of Live Amphibians and Reptiles in Field Research*, and the *Guidelines to the Use of Wild Birds in Research*.

II. Institutional Official

II.1. The Director of Research is the Institutional Official responsible for the Animal Care and Use Program. The Vice President for Research and Provost assist in overseeing the Animal Care and Use Program. The Institutional Official has appointed an Institutional Animal Care and Use Committee (IACUC) and alternates, which is responsible for oversight and evaluation of the animal care and use program, its procedures and facilities to ensure that they are consistent with the recommendations of the Guide, AWR, PHS Policy, and IACUC Policies.

III. Institutional Animal Care and Use Committee

III.1. IACUC members (current and new) attend an orientation/training by the IACUC Chair regarding the functions of the IACUC, and the regulations, policies and procedures that govern the University's Animal Care and Use Program, as itemized in PHS Policy IV.B.1-8.

III.2. The IACUC has authority to review, approve, or require modification in order to secure approval, or withhold approval of proposed research or teaching using animals, review the facilities and program for animal care and use, including laboratories outside of animal facilities where procedures are performed, review and, if warranted, investigate concerns involving the care and use of animals, prepare written reports of its evaluations, make recommendations to the Institutional Official concerning any aspect of the animal care and use program, and suspend any activity involving animals that does not conform to the Guide, AWR, PHS Policy and/or the IACUC Policies.

III.3. The IACUC consists of not less than five members, and includes a Doctor of Veterinary Medicine with training or experience in laboratory animal science and medicine who has direct or delegated program responsibility for activities involving animals at the institution, a practicing scientist experienced in research involving animals, a member who is not affiliated with the University other than as a member of the IACUC and is not a member of the immediate family of a person who is affiliated with the University, and who represents general community interests and a member whose primary concerns are in a nonscientific area.

III.4. IACUC members and alternates are appointed by the Institutional Official, and are listed on the IACUC rosters submitted to the PHS. A specific designation of IACUC members and their alternates is made to ensure that the committee is properly constituted at all times (as described above). An alternate may not contribute to the formation of a quorum or vote unless the member for whom they substitute is not available. Alternates receive the same training and orientation as IACUC members, and are expected to "vote their conscience" as opposed to representing the position of the member they are replacing.

III.5. All vertebrate animal use, including field studies, conducted by University faculty, students, or staff, or supported by University funds, must be reviewed and approved by the IACUC on either an *Animal Use Application Form*, or *Animal Use Application Form – Wildlife Studies*, prior to the initiation of that activity, regardless of where it will be performed.

III.5.A. The IACUC reviews submitted protocols either at a convened meeting of a quorum (simple majority), or through the use of designated reviewers. Designated reviewers are appointed by the Chair.

III.5.B. The use of designated reviewers occurs only after the entire IACUC is provided with a list of the protocols to be reviewed, and each member is provided an opportunity to call for full committee review of any protocol. If a full committee review is not requested, at least one or more members of the IACUC, designated by the chair and qualified to conduct the review, reviews the protocol and has the authority to approve, require modifications, or request full committee review. The IACUC Chair may also request a review by a consultant reviewer(s) who may have more expertise than any available committee member for the purpose of providing an informed, objective non-biased review of the animal use protocol. Consultants may not approve or withhold approval of an activity or vote with the IACUC unless they are also members of the IACUC.

III.5.C. In either review setting the animal user is provided with comments, questions or concerns of the committee in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval. If the IACUC decides to withhold approval of an activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or in writing to the committee. The responses are distributed to the committee or designated reviewer and a decision of

approval is usually issued within 10 working days. See Appendix 8 for more information regarding the designated review process.

III.6. Minor changes to the IACUC-approved protocol must be described on the *Amendment to Animal Use Application Form*.

Some examples include:

- A change in the certified research personnel
- A change in the title
- A change in the funding source
- An addition of another strain of the same species if justified in writing
- Up to a 20% increase in the number of animals available to the protocol if justified in writing

Amendments to existing protocols are reviewed by the IACUC Chairperson within 7 days of receipt, and then by the full IACUC membership at its next regular meeting. Minor procedural changes can be proposed to the IACUC at its next regular meeting, but must be justified in writing, within the scope of the original research hypothesis, involve the original species, and remain within the original Category of Research.

III.7. Major changes in research, teaching, or testing protocols using animals cannot be amended to an existing IACUC-approved protocol, but must be described on a new *Animal Use Application Form*, or *Animal Use Application Form – Wildlife Studies*. After three years all continuing studies must be completely re-described in a new application to the IACUC.

III.8. IACUC members and alternates are required to attend each IACUC meeting. Each *Animal Use Application Form*, or *Animal Use Application Form – Wildlife Studies*, is presented by a primary and/or secondary reviewer (IACUC members), who after presenting their findings regarding the application, proposes a motion to either approve, approve pending minor clarification, or disapprove the application. After discussion, the motion is seconded, and the full IACUC committee votes. If an IACUC member present at the meeting is listed as a Principal Investigator (PI) on a animal use form being reviewed by the committee, they will be asked to leave the meeting room during the voting process.

III.9. If activities involving animals are to be conducted at another institution by University personnel, or supported by University funds, that institution must provide the IACUC with a letter on official letterhead that indicates they are anticipating the presence of the research protocol, that they have an assurance on file with the PHS, and that their IACUC has approved the proposed animal use. In order to ensure clear definition and understanding of the planned collaboration, whenever the collaborating institution has agreed to perform a significant portion of the animal use aspects of a

research grant or contract awarded to the University, the University's IACUC should be provided with written evidence that the collaborating institution's IACUC has approved the activity. These documents must be received from the collaborating institution prior to initiating any work. In addition, the IACUC must be informed of any issues raised by the collaborating institution's IACUC during their inspection of the activity, program, or facility while hosting the research activity.

III.10. Animals not described in an IACUC-approved *Animal Use Application Form*, or *Animal Use Application Form – Wildlife Studies*, are not permitted within University research or teaching laboratories.

III.11. If funding is to be obtained, then the title of an IACUC application must match the title of the grant or contract that supports the proposed research activity involving animals. If an activity involving animals that is described in an IACUC-approved protocol is supported by multiple grants with different titles, or a grant is awarded later during the 3-year approval period of the IACUC protocol that supports the same approved activity involving animals, the PI must inform the IACUC by completing and submitting the *Amendment to Animal Use Application Form*. A single IACUC protocol can be used to represent the animal use activities of multiple grants or contracts if the IACUC-approved protocol is amended with all applicable titles and sources of support, and the described activity involving animals in the IACUC-approved protocol is identical to the activities described in each of the grants identified.

III.12. If test substance(s) that are potentially hazardous are to be administered to animals, prior authorization of use of the test substance(s) by the appropriate Safety Committee is required before approval by the IACUC. Approval of the IACUC application involving hazardous materials is contingent on a pre-performance meeting involving staff that represent the applicant's laboratory, the Animal Facility Manager, the IACUC, and the appropriate Safety Committee(s). This pre-performance meeting is required in order to ensure that all involved personnel are aware of the precautions, containment practices, facilities, protective devices, disposal and decontamination procedures, and other necessary safety procedures that must be followed to protect personnel, and prevent accidental animal exposure to the hazardous material.

III.13. The IACUC conducts periodic audits of active animal use protocols, and inspects laboratories outside of the animal facilities where animals are used. These audits and inspections serve as an additional review of the effectiveness of the animal care and use program, and are initiated during each semi-annual inspection of facilities and program by the IACUC, or whenever necessary. These audits and inspections ensure that sufficient animal care and clinical oversight is provided and recorded, that animal pain, distress, or discomfort are anticipated, avoided, or alleviated, that work areas are uncluttered and adequately decontaminated, that current supplies and procedures are used, that appropriately decontaminated instruments are used, and that the risks of all hazards are minimized. In determining which protocols to audit and laboratories to inspect, the IACUC is especially interested in ensuring the good practices of protocols involving Category E procedures, survival surgery, the administration or use of hazardous materials, or the use of scheduled substances.

III.14. The IACUC reviews the animal care and use program and inspects animal facilities, and laboratories outside of facilities where animals are used at least every 6 months. During these reviews and inspections the Semi-Annual Program and Facility Review Report is completed. Deficiencies identified are classified as either “major” (i.e., those which affect animal welfare), or “minor”, and a schedule by which corrections will be accomplished is assigned. Each semi-annual report is reviewed and signed by a majority of IACUC members and submitted to the Institutional Official for his review and signature. Each report includes changes made to the program and improvements made to facilities. Any minority views if given will also be included with this report.

III.15. Non-members of the IACUC are welcome to attend IACUC meetings. Questions regarding animal care and use can be submitted to the IACUC Coordinator at telephone 407-882-1164, fax 407-823-3299, or email IACUC@mail.ucf.edu.

IV. Office of Research Regarding Animal Care and Use

IV.1. The Office of Research provides the IACUC with administrative support services, and assists the IACUC with its functions of oversight and evaluation of the animal care and use program.

IV.2. The Office of Research maintains files of IACUC protocols, each of which is initiated by an *Animal Use Application Form*, or *Animal Use Application Form – Wildlife Studies* or an *Amendment to Animal Use Application Form*, and notifies applicants of IACUC actions.

IV.3. Applicant PIs must respond to all communications from the Office of Research regarding IACUC deliberations promptly and no later than the designated time frame in the communication. Failure to respond within the designated time will result in the Office of Research informing the IACUC of this non-response and recommending administrative closure of the study.

V. Occupational Health and Safety

V.1. The Manager of the Animal Facility provides all personnel who will be working with animals with information regarding health monitoring, potential zoonoses, and health assessments and immunizations relating to their animal contact.

V.2. Information regarding potential zoonosis, and practices of personnel hygiene, which limit exposure and risk of contracting zoonosis are posted on the corridor wall of each of the facilities. The nature of noxious, toxic, hazardous, infectious or carcinogenic agents or compounds when used, are posted on the door of the room containing the animal collection exposed to such agents.

VI. Personnel Training and Experience

VI.1. All personnel should have adequate knowledge and experience to perform their duties of animal care, use, and treatment.

VI.2. All personnel should be sufficiently familiar with the AWR, PHS Policy, Guide, and these IACUC Policies, so that their care, treatment and use of animals will be in accordance with these principles.

VI.3. Annually, all research personnel should be listed on a *Request for the Annual Re-Certification of Research Personnel using Animals*, which the PI submits to the IACUC.

VI.4. All new personnel should contact the animal facility manager so that a general orientation and protocol-specific, and/or species-specific training can be arranged.

VI.5. The veterinary staff can coordinate additional assistance with species-specific or protocol-specific techniques whenever additional individual instruction is requested, and/or required by the IACUC.

VI.6. All animal care program staff are required to prepare for and receive certification by the American Association for Laboratory Animal Science (AALAS).

VII. Reporting Deficiencies in Animal Care or Treatment

VII.1. Deficiencies in animal care, use, or treatment are reported to: Cristina Caamano, IACUC Coordinator at (407-882-1164), Norman Guilloud, attending veterinarian (908-797-5970), Farol Tomson, IACUC Chairperson (352-371-7030), Teresa Krish, TAF Manager (407-823-2918) or Robert Banks, WAF Manager (407-823-4098), or Thomas O'Neal, Institute Official (407-882-1120).

VII.2. This reporting-feedback mechanism of observations made regarding the practices of animal care and use within these laboratories, contributes an important oversight, and assists in the continuous development of the animal program.

VII.3. Such reports, suggestions, complaints, or compliments are made with complete protection of the reporting individual from any discrimination or reprisal.

VII.4. The IACUC deliberates the reported alleged deficiency at its next regularly scheduled meeting, or the IACUC Chairperson can call an emergency quorum to discuss the issue in advance of a regular meeting if deemed necessary. The IACUC Chairperson can choose to invite involved personnel to the meeting to whom questions can be directed. The IACUC reports the findings of its deliberations to the PI and the Institutional Official.

VIII. Suspension of Animal Use Privileges

VIII.1. The IACUC may suspend an activity that it previously approved if it determines that the activity is not being conducted in accordance with the AWR, the Guide, or these IACUC Policies or when the activity does not match the description of the activity originally approved by the IACUC.

VIII.2. The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with a suspension vote of a majority of the quorum present.

VIII.3. If the IACUC suspends an activity involving animals, the PI will be informed in writing of the suspension, its conditions, and the expectations of the IACUC, which need to be met before additional activities involving animals resume.

VIII.4. If the IACUC suspends an activity involving animals, the Institutional Official, in consultation with the IACUC, shall review the reasons for the suspension, take appropriate corrective action, and report that action with a full explanation to USDA/APHIS and PHS/OLAW, and the agency funding that activity.

VIII.5. In the event that an IACUC-approved protocol expires, or is closed by the PI, or for any reason animals which had been maintained under a formerly approved protocol remain within the laboratories, but are not described in an IACUC-approved protocol, the Office of Research will serve written notification of non-compliance to the PI, and request that the PI either submit a new application for animal use, identify the IACUC-approved protocol which now describes the use of these animals, or formally request in writing that the closed protocol be reopened and reviewed. Absence of a written response to this written notification of noncompliance in excess of thirty days may result in the removal, re-assignment, or euthanasia of the subject animals.

VIII.6. The PI is responsible for ensuring that adequate fiscal support has been retained, and is available for the procurement, care, and use of all animals registered under their IACUC-approved protocol(s) in their name.

VIII.7. Since the PI has assured the IACUC on their *Request for the Orientation and Certification of New Research Personnel Using Animals* that the research personnel contributing to their IACUC-approved protocols have been provided adequate training, and have adequate knowledge and experience to perform their duties, the PI is notified whenever the performance of their staff is not in accordance with these IACUC Policies. The PI should ensure that any corrective retraining or actions, required of them or their staff by the IACUC, in response to deficiencies in animal care or use are accomplished.

VIII.8. Inadequate animal care or use, inadequate clinical animal oversight or treatment, inadequate anticipation or alleviation of animal discomfort, distress, or pain, inadequate animal medical record keeping, or conducting procedures involving animals prior to their approval by the IACUC can result in the suspension of animal use privileges of the supervising PI and involved research or animal care staff.

VIII.9. The IACUC deliberates the reported alleged deficiency at its next regularly scheduled monthly meeting, or the IACUC Chairperson can call an emergency quorum to discuss the issue in advance of a regular meeting if deemed necessary. The IACUC Chairperson can choose to invite involved personnel to the meeting to whom questions can be directed. The IACUC reports the findings of its deliberations to the PI and the Institutional Official.

VIII.10. After its review of the reported alleged deficiencies in animal care or use, the IACUC may elect to require additional training of the involved personnel and/or may elect to suspend or revoke the animal use privileges of that individual and/or PI. Personnel may be required to work only in the presence of another qualified employee, may need to complete additional training, may need to assist with an audit of research and animal medical records, and/or may need to undergo an evaluation of research technique. All actions made by the IACUC regarding individuals will be reported to them in writing, with a copy to their supervising PI and the Institutional Official.

IX. Transportation, Relocation, or Reassignment of Animals

IX.1. All transportation of animals should occur only when any risks of exposure to environmental extremes, crowding, infectious agents, and possible zoonoses are minimized. Any movement of animals should be reviewed with the Animal Facility Manager BEFORE the move.

IX.2. Transporting of animals to separate housing in research laboratories, procedural, or testing areas outside of an animal facility may jeopardize the integrity of research data, impairs regulatory oversight, and abrogates the implementation of uniform standards of animal care and use within those areas.

IX.3. Movement of animals within animal housing rooms is discouraged. Movement of animals between animal housing rooms of a single facility, or between separate facilities is discouraged.

IX.4. Transfer of unused animals from one IACUC-approved use to another is permitted, providing the involved protocols have already described the transfer, or after the necessary amendments have been approved by the IACUC.

IX.5. Shipment of animals to or from other institutions must be done in such a way to minimize or prevent the introduction of disease to resident animals. Veterinary involvement at both ends is usually required.

IX.6. Animals received by the UCF Animal Care Facility must be from approved vendors and be accompanied by health certificate papers ensuring that the animals received are free from transmittable organisms or diseases.

IX.7. Once an animal housed within the UCF Animal Care Facility is removed, it will not be allowed to be returned to the Animal Care Facility.

X. Avoidance and Alleviation of Research Animal Pain and Discomfort

X.1. The IACUC affirms that the study and use of animals contributes to the advancement of knowledge, and that potential for research animal pain, discomfort, or distress must be minimized.

X.2. Pain is a perception of suffering or agony that results from mechanical, thermal, or chemical stimuli acting on nociceptors, which in turn generate impulses via neural pathways, mediating spinal reflexes and central sensory processing by the reticular formation, thalamus, and cerebral cortex. Discomfort or distress is considered to refer to those conditions that are not in themselves painful, but which are disagreeable, and which the animal would normally choose to avoid.

X.3. Although animals that are in pain may not behave like humans, (e.g., pain in animals may be accompanied by immobility and silence, in contrast to the groans and cries of human patients), it is assumed that procedures that cause pain in humans cause pain in animals.

X.4. The presence of pain in animals can be recognized by alterations in animal behavior (e.g., reduced activity, reduced grooming, hunched-up posture, altered gait, changes in temperament, vocalizations, reduced food and water intake, reduced urinary and fecal output), and in physiological variables, (e.g., reduced depth of respiration, increased heart rate, and reduced hydration status).

X.5. Animal pain and discomfort can produce a range of undesirable physiological changes, which may radically alter measured responses to experimental stimuli, as well as the rate of recovery from surgical procedures, hence, its avoidance and alleviation are in the best interest of both the animal and researcher.

X.6. Reducing post-procedural/post-operative pain and discomfort is accomplished by good nursing care, (e.g., keeping the animal warm, clean, dry and well padded), and by the administration of analgesic drugs.

X.7. The selection of an appropriate analgesic involves consideration of the level of animal pain anticipated or presumed, the species involved, and the experimental protocol. Severe pain, such as may occur during the post-operative period, can be alleviated by the administration of narcotic analgesics, (e.g., buprenorphine, an opioid partial agonist). Non-steroidal anti-inflammatory drugs, (e.g., aspirin), with or without the infusion of local anesthetics, can control mild to moderate pain in some species.

X.8. In addition to the avoidance and alleviation of pain and discomfort, adequate post-procedural /post-operative animal care also includes efforts to prevent and/or treat post-anesthetic complications, (e.g., aspiration, hypostatic pneumonia, cardiovascular and respiratory depression, dehydration, and infection).

X.9. The IACUC maintains an inventory of animal use regarding the potential for pain or discomfort. In their application for animal use, PIs designate the described animal use to

one of three categories of research. Research Category C involves procedures, which produce momentary, slight, or no pain, discomfort or distress. Research Category D involves procedures, which produce more than momentary or slight pain, discomfort or distress, which is alleviated by the use of appropriate anesthetics/analgesics. Research Category E involves procedures, which produce pain discomfort, or distress, which cannot, or is not alleviated by the administration of appropriate anesthetics/analgesics.

X.10. When proposing Research Categories D and E activities involving animals where painful or stressful outcomes are anticipated or possible, the PI must define in writing the clinical criteria which will be used to ensure timely intervention and treatment, or removal of the animals from the study, either in advance of, or immediately after recognition of the discomfort, or the specific clinical end point at which humane euthanasia of the animals will be accomplished. The earliest possible clinical endpoint that will contribute to the resolution of the hypothesis must be identified and utilized. If avoidance or alleviation of animal pain or discomfort adversely affects the protocol, the PI must provide a detailed justification of why treatments cannot be initiated.

X.11. The PI and associated research staff should maintain written records of activities whenever painful or stressful outcomes are anticipated or possible. Records should be kept within the animal facility with entries that describe when the painful or stressful outcome is first recognized, what treatments are instituted, and when the discomfort is resolved, or when the animal is humanely euthanized.

X.12. The written justification for the use of animals involved in Research Category E procedures must identify and utilize the earliest possible clinical end point, which will contribute to the resolution of the hypothesis. If death is determined to be the earliest possible endpoint, which will contribute to the specific aims of the research, then a written justification of why an earlier clinical endpoint is inadequate for resolving the proposed hypothesis must be included in the IACUC application.

X.13. Investigators must consider the needs and well-being of animals involved in protocols that have a potential to cause animal discomfort, pain, or distress which may not be reliably anticipated or controlled, including those of new or unique phenotypes of transgenic, knock-out, knock-in, or genetically-mutant rodents, and provide written assurance of adequate clinical oversight and intervention criteria to prevent animal discomfort, or a justification of why intervention conflicts with the proposed investigation.

X.14. Investigators must continuously refine methods so as to avoid, alleviate and/or minimize any animal pain or discomfort associated with experimental protocols, and search for alternatives, which reduce animal use.

X.15. Investigators and their staff must conduct clinical and post mortem investigations whenever animals experience morbidity or mortality not anticipated in the protocol. Investigators and their staff must make entries to nonrodent mammalian medical records, which summarize the clinical diagnostic and necropsy findings of an unanticipated animal morbidity or mortality, which occurs unrelated to the protocol, so that research methods can be refined.

XI. Anesthesia and Analgesia

XI.1. In designing an experimental protocol where pain is anticipated, the principal investigator is obligated to consult a veterinarian regarding anesthesia and/or analgesia. The principal investigator is compelled to institute adequate practices of anesthesia and analgesia, unless the protocol precludes such practice (Category E research), the investigator has justified such in writing, and the IACUC has approved such practice.

XI.2. Neonatal animals have low energy reserves. Periods of hypoxia can deplete limited hepatic glycogen stores and result in hypoglycemia. Neonates have a reduced capacity to detoxify a wide range of drugs. It is preferable to use inhalation anesthetics so that recovery is rapid and normal feeding is resumed as soon as possible. Although the deliberate production of hypothermia has been shown to produce consistent and safe anesthesia for minor surgical procedures with rapid induction and recovery times in neonatal rodents <4 days of age, inhalation anesthetics should be used in rodents >4 days of age, or during major surgical procedures.

XI.3. Cooling ectothermic species (e.g., frogs) to 4°C will decrease their metabolism and facilitate their handling, but there is no evidence that whole body cooling reduces pain or is clinically efficacious as an anesthetic.

XI.4. An abbreviated list of useful drug dosages is attached.

XII. Controlled Substances

XII.1. The procurement, distribution, use, security, and record keeping of controlled substances are regulated by the Drug Enforcement Administration (DEA) and are guided by the regulations detailed in 21 CFR 1300-1308.

XII.2. Every person conducting animal research activity with a controlled substance is required to register with the Drug Enforcement Agency (21 CFR, 1301.21). Registration is required annually and there is no fee for state employees. Each principal investigator working with DEA controlled substances will be responsible for registering with the DEA and for assuring compliance with applicable state and federal regulations.

XII.3. The Attending Veterinarian can assist principal investigators in complying with applicable rules and regulations. This will include educating researchers about the requirements, assisting them as necessary during implementation, and providing regular oversight to insure compliance is being maintained.

XII.4. Both the state and federal law classify controlled substances into five categories according to their medical use and potential abuse. For example, Schedule I substances are categorized as having no medical value and having the highest potential for abuse. Schedule V is categorized as having the least potential for abuse.

XII.5. For substances in Schedule II-V, one should submit DEA Form 225. Orders for Schedule I or II substances must be in writing and accompanied by DEA Form 222. Separate registrations may be required for research use (II-V), institutional use (II-V), research or instructional use (I). Please consult section 1301.22.

XII.6. For Schedule I substances, in addition to DEA Form 225, the applicant is required to submit 3 copies of a research protocol (21 CFR, 1301.33) including:

- * Investigator - Name, address, institution and a qualifications statement including a curriculum vitae with bibliography.
- * Project - Title, statement of purpose, controlled substance name and amount needed, location of research, security statement and a technical description of the substance use.
- * Authority - Institutional approval and grant number if applicable.

XII.7. On August 12th, 1999, Ketamine was included into the Federal Drug Enforcement Regulations. Purchase, use, and disposal of Ketamine requires a Schedule III permit. All other DEA requirements for Schedule III will apply to Ketamine.

XII.8. DEA PHONE NUMBERS and CONTACTS in Orlando:

1-407-333-7046 Randy Rine or Sharon Lynne

In Washington, D.C.: 1-800-882-9539 or 1-202-307-7255

XII.9. DEA Controlled Substance Summary

1. Each PI must have own DEA permit.
2. All DEA controlled substances must be stored in a locked cupboard (not a glass-door cupboard, nor in a locked box in an unlocked drawer/cupboard).
3. Inventory and use log for each DEA controlled substance must be kept from initial purchase to final use or disposal.
4. All purchase orders (222's), loss records and disposal records must be kept on file.
5. An "Authorized Person Questionnaire" must be filled out and kept on file for each person working with DEA controlled substances under the DEA permit holder's supervision.

XII.10. Noncompliance can result in the suspension of privileges to use controlled substances and animals, in accordance with IACUC Policies X.

XIII. Surgical Techniques and Post-operative Care

XIII.1. Non-rodent mammalian survival surgical procedures must be conducted within veterinary supervised facilities for non-rodent surgery, where separate areas are provided for pre-operative animal preparation, surgeon's pre-operative scrub, the operating room, and for post-operative monitoring and care of the involved animal(s).

XIII.2. All survival surgical procedures are conducted using aseptic technique.

XIII.3. The surgeon must wear sterile gloves, gown, cap and mask when conducting major, survival, nonrodent surgical procedures. A sterile gown and cap are recommended when conducting rodent surgical procedures. Sterile gloves are required, and must be changed between all animal surgeries, or if non-sterile surfaces are handled.

XIII.4. Rodent and nonmammalian surgical procedures are conducted in a designated, uncluttered area, on a benchtop or hood surface top, which has been cleaned and treated with disinfectant prior to any procedure. As a work surface disinfectant prior to placing the animal on the work area, the surgeon may use clidox, chlorox, quatricide-PV, or quaternary ammonia. Alcohol as a sole disinfectant of the work surface is not appropriate. The surrounding work surface is draped with sterile draping, upon which instruments are placed.

XIII.5. Sterile instruments are used for all survival surgical procedures. Acceptable methods of instrument sterilization include autoclave, instrument tips flamed with 95% alcohol, a glass bead sterilizer and cooled with sterile saline, or a benz-all, or amerse instrument sterilant solution. Sterilization of instruments using cold sterilants or a glass bead sterilizer and cool saline are acceptable between surgeries when multiple rodents are involved in surgeries. Instruments must be kept on a sterile drape, pan or tray during the procedures. Soaking in alcohol is not an acceptable means of decontaminating surgical instruments.

XIII.6. Preparation of the patient and surgical site often includes fasting, shaving, a surgical scrub with Betadine soap, spraying with alcohol, spraying with Betadine solution, and draping of the animal with sterile drapes as appropriate. The skin of scaled terrestrial vertebrates can be prepared with dilute Betadine, Nolvasan, 10% chlorhexadine, or 70% ethanol. The skin of amphibian and teleost vertebrates contain glands which produce antimicrobial substances, and is delicate and easily damaged. Consequently, risks associated with the use of topical skin disinfectants with these species outweigh their potential benefits and their use is not recommended.

XIII.7. Procedures in which pain or discomfort is anticipated or perceived post-operatively must include intra-operative or the immediate post-operative administration of analgesics. In-date anesthetics and analgesics must be used. If volatile anesthetics are used, appropriate scavenging must be in use.

XIII.8. Body cavities are typically closed in at least two layers, with an absorbable inner layer(s), and a nonabsorbable skin layer, or absorbable subcuticular layer. Holding tissue layers must be sutured in an interrupted pattern. Nonabsorbable skin sutures or

staples must be removed 7-10 days post-operatively. Absorbable suture material used in deep tissue closure for nonmammalian vertebrates should be those that are absorbed by hydrolysis (e.g., polydioxone or polylactin 910), and not those that rely on proteolysis (e.g., chromic catgut) which induce inflammation and have a prolonged presence postoperatively.

XIII.9. Post-operative recovery must be on heated pads or in an otherwise warm environment, and under direct supervision until full recovery from anesthesia (i.e., sternal recumbency and intentional movement).

XIII.10. Surgical records describing surgical events involving nonrodent mammalian species must be kept by the PI. Log entries must at least include a pre-operative assessment, an anesthetic plan, records of the induction and monitoring of general anesthesia, a brief description of the surgical procedures performed, an intraoperative assessment, a record of the recovery from anesthesia (or method of euthanasia of the anesthetized animal), a post-operative assessment, and any complications.

XIII.11. Post-operatively, the heart or pulse rate, respiratory rate, mucous membrane color or capillary refill time, and body temperature of nonrodent mammals must be recorded in daily log form at least once each between post-operative days 1-3. A daily entry by the PI or research staff must be made in the medical records of post-operative nonrodent mammals until suture removal or the fifth post-operative day whichever is longer, which indicates that the attending research clinicians have assessed their patient animals, including anticipating and alleviating post-operative pain or discomfort, and provided timely and appropriate responses to clinical abnormalities, and any necessary treatments. In addition, the dose and route of all post-operative analgesics, antibiotics and treatments, and the date of skin suture removal should be noted.

XIII.12. Post-operative care includes appropriate analgesia and nursing care, monitoring physiological functions, behavior and for any complications, and complete record keeping.

XIV. Multiple Major Survival Surgical Procedures

XIV.1. Major surgery penetrates and exposes a body cavity (e.g., laparotomy, thoracotomy, craniotomy), or produces impairment of physical or physiologic function (e.g., joint replacement, limb amputation).

XIV.2. Multiple major survival surgical procedures on a single animal are discouraged but may be permitted if scientifically justified by the applicant and approved by the IACUC.

XIV.3. Multiple major survival surgical procedures may be justified if they are related components of a research project, if they conserve scarce animal resources, or if they are needed for clinical reasons. In most cases, cost savings is not an adequate reason for performing multiple major survival surgical procedures.

XIV.4. If multiple major survival surgical procedures are approved by the IACUC, particular attention must be provided by the research staff to animal health and well-being through frequent and continuing evaluations.

XIV.5. Multiple partial ovariectomies involving *Xenopus* frogs is permissible if scientifically justified, perhaps in part due to the need to identify individual frogs that produce oocytes of sufficient quality for the proposed study. As an example, oocytes from different frogs can have quite different efficiencies of DNA transcription or RNA translation, and this quality is rather constant over time. Consequently, it may be desirable to identify those individual frogs that produce acceptable quality oocytes for the planned transcriptional or translational assays, and reharvest oocytes from those frogs. If such an approach is proposed and scientifically justified to, and approved by the IACUC, no more than 4 partial ovariectomies may be performed on an individual frog, and a minimum 3-week inter-operative interval must be allowed. Records must be kept that identify the individual frogs involved, and the dates and number of surgeries performed on each frog.

XV. Animal Identification and Medical Records

XV.1. Adequate animal care includes adequate animal medical record keeping. Although veterinary, animal care, and husbandry staff may make contributions to research protocols involving animals, the PI and associated research staff named on an IACUC-approved protocol serve as the primary attending clinicians of all animals housed on behalf of that protocol. As such, research staff are responsible for providing adequate clinical oversight, and post-operative or post-procedural care of the animals, for anticipating and alleviating animal pain or discomfort whenever possible, and for maintaining complete animal medical records, with entries made in sufficient detail and at intervals specified by these IACUC Policies.

XV.2. Animals are identified on cage cards as to, the requesting PI, the IACUC file #, date of arrival, source, and physical findings, including species, strain, sex, weight or age, and should include any identifying features, and/or permanent markings.

XV.3. The *Nomenclature Rules and Guidelines* for identifying or naming mouse lines or strains of the International Committee on Standardized Genetic Nomenclature for Mice viewable at <http://www.informatics.jax.org/> should be followed.

XV.4. All animals should be acclimated a minimum of seven days prior to use (depending on the vendor involved and the room where animals are being housed).

XV.5. Individual animal medical records are maintained by the PI and associated research staff. Individual animal medical records of all nonrodent mammals must at least include an *Arrival Status* form that describes the condition and characteristics of each animal upon arrival, and *Progress Notes* forms that record items described below in IACUC Policy XIX.5.

XV.6. Log entries that must be made by the PI and research staff on *Progress Notes* forms include describing all procedures, substance administrations, tissue collections, observations, treatments, or uses involving nonrodent mammals. Procedures or assessments involving nonrodent mammals that are approved by the IACUC must be performed and recorded by the PI and research staff at intervals indicated in the approved protocol. Logs must be kept by the research staff in the animal facility.

XV.7. Nonrodent mammalian medical records must also include weekly entries made by the research staff on *Progress Notes* forms, which at least summarize, an impression of overall condition, food and water intake, and voidings, any clinical abnormalities or complications, any treatments administered in response to observed abnormalities, and any experimental procedures.

XV.8. In addition, nonrodent mammalian medical records must include a monthly clinical entry re-characterizing the condition of the animal, conducted by the veterinary and animal care staff that includes the animal's body weight, body temperature, heart or pulse rate, respiratory rate, and mucous membrane color or capillary refill time, which is logged on *Progress Notes* forms.

XV.9. When clinical abnormalities are recognized in nonrodent mammals, the PI and research staff must make entries in the medical record, which at least document, the current body weight, body temperature, heart or pulse rate, respiratory rate, and mucous membrane color or capillary refill time, the clinical abnormality observed, the laboratory, diagnostic findings, and the treatments initiated, and the consequences to treatment.

XV.10. Log entries describing surgical events involving nonrodent mammalian species must be kept by the PI in the animal room/facility. Log entries must at least include a pre-operative assessment, an anesthetic plan, records of the induction and monitoring of general anesthesia, a brief description of the surgical procedures performed, an intraoperative assessment, a record of the recovery from anesthesia (or method of euthanasia of the anesthetized animal), a post-operative assessment, and any complications, treatments, and/or plans.

XV.11. Post-operatively, the heart or pulse rate, respiratory rate, mucous membrane color or capillary refill time, and body temperature of the nonrodent mammal must be recorded in the medical log on a *Progress Notes* at least once each between post-operative days 1-3. A daily entry by the PI or research staff must be made in the medical records of post-operative nonrodent mammals until suture removal or the fifth post-operative day whichever is longer, which indicates that the attending research clinicians have assessed their patient animals and have provided any necessary or required treatments. In addition, the dose and route of all post-operative analgesics, antibiotics and treatments, and the date of skin suture removal must be noted.

XV.12. The PI and associated research staff should maintain written records of activities whenever painful or stressful outcomes are anticipated or possible in any animal. Records should be kept within the animal facility on forms, with entries that describe

when the painful or stressful outcome is first recognized, what treatments are instituted, and when the discomfort is resolved, or when the animal is humanely euthanized.

XV.13. Unanticipated clinical abnormalities or complications in any animal must be resolved through the cooperative interaction of research, animal care, and veterinary staff.

XV.14. Research staff must make entries to nonrodent mammalian medical records that summarize the clinical diagnostic and necropsy findings of an unanticipated animal morbidity or mortality that occurs unrelated to the protocol, so that research methods can be refined.

XV.15. A copy of each individual animal medical record that clearly indicates the final disposition of the animal, must be filed with the facility manager.

XV.16. Inadequate animal care, or inadequate animal medical record keeping can result in the suspension of animal use privileges in accordance with IACUC Policy X.

XVI. Animal Euthanasia

XVI.1. Euthanasia is the induction of humane death without pain, anxiety, or distress. Acceptable techniques safely result in rapid animal unconsciousness, cardiac and respiratory arrest, and loss of brain activity.

XVI.2. Anxiety and distress can be minimized, and safety assured by careful handling, calming, and gentle restraint, and by the appropriate selection of, and training and experience in euthanasia technique suitable to the research protocol and the species used.

XVI.3. Acceptable, conditionally acceptable, and unacceptable euthanasia agents, methods and techniques have been described by the *2000 Report of the American Veterinary Medical Association Panel on Euthanasia*, JAVMA 218(5):669-696, 2001. These recommendations provide the basis for acceptable euthanasia techniques and are adopted as such. These recommendations and those in the *Acceptable Field Methods in Mammalogy*, the *Guidelines for the Capture, Handling, and Care of Mammals*, the *Guidelines for Use of Fishes in Field Research*, the *Guidelines for Use of Live Amphibians and Reptiles in Field Research*, and the *Guidelines to the Use of Wild Birds in Research* provide the basis for acceptable euthanasia techniques of wild, avian, aquatic, and ectothermic animals, and are adopted as such. Specific policies regarding historically common euthanasia techniques follow.

XVI.4. Ether is both flammable and combustible. Ether inhalation is unpleasant and irritating, can cause profuse bronchial and salivary secretions, coughing, and laryngospasm. Ether inhalation is not safe to use within these laboratories, and can be substituted by the use of halothane, if an inhalant anesthetic is required.

XVI.5. Decapitation is an acceptable method of euthanasia of sedated or anesthetized adult rats and other small animals. Cervical dislocation is an acceptable method of euthanasia of sedated or anesthetized rodents <200 gms in body weight.

XVI.6. Choral hydrate is only acceptable for euthanasia of deeply anesthetized large animals, and only when administered intravenously. Choral hydrate is not acceptable for small animals.

XVI.7. Chloroform is hepatotoxic, cardiotoxic, and nephrotoxic, is an unacceptable method of euthanasia, and should be substituted with other agents, methods or techniques of euthanasia.

XVI.8. Compressed carbon dioxide in gas cylinders is the only acceptable source of carbon dioxide for euthanasia. Carbon dioxide inhalation is an acceptable method of rodent euthanasia, provided the chamber is prefilled with CO₂, and provided that CO₂ narcosis is followed by the assurance of the cessation of cardiovascular and respiratory movements, and preferably, subsequently followed by thoracotomy. CO₂ euthanasia is not an acceptable technique with rabbits or larger animals. Carbon dioxide generated by other means such as dry ice or antacids is unacceptable, if animals come into contact with the ice or the liquids.

XVI.9. The intravenous injection of a barbituric acid derivative is a preferred method of euthanizing animals. Adequate pentobarbital euthanasia dosage for all species is 80 mg/kg.

XVI.10. Exsanguination is not used as a sole means of euthanasia. Animals may be exsanguinated when surgically anesthetized.

XVI.11. Neonates are resistant to hypoxia. Consequently, inhalants should not be used alone as a sole means of euthanizing neonates. Inhalants may be used to induce unconsciousness, followed by another method of euthanasia to ensure death. Euthanasia of feti or prenatal mice should be accomplished immediately after removal from the dam. Incomplete neural development in mouse feti less than 14 days of gestation suggests that pain perception at this age is minimal.

XVI.12. Cooling ectothermic species (e.g., frogs) to 4°C will decrease their metabolism and facilitate their handling, but there is no evidence that whole body cooling reduces pain. Hypothermia prior to physical methods of euthanasia, or hypothermia alone are unacceptable methods of euthanasia.

XVI.13. Following all methods of euthanasia, animal death is assured by the determination of the cessation of cardiovascular and respiratory movements.

XVII. Animal Acclimation and Conditioning

XVII.1. Investigators are encouraged to use specific pathogen free animals - whenever available - for research and teaching, and recognize that latent disease conditions complicate and interfere with the interpretation of research data.

XVII.2. All animals should be acclimated a minimum of seven days prior to use. Animal conditioning should be implemented whenever animals with unspecified clinical history or health status are requested.

XIII. Animal Facilities

XIII.1. All facilities for University of Central Florida animal care and use are listed on the registration #58-R-0016 with the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS).

XIII.2. Each college or department which hosts a facility for animal care and use is responsible for all physical structural maintenance and repairs, and all major equipment maintenance, repairs, and replacements within their respective facilities, including, but not limited to exterior roof, walls and doors, utilities, lighting, heating/ventilation/air conditioning, interior wall/floor/ceiling surfaces and doors, cage washers, sterilizers, boilers, steam generators, water conditioners/softeners, animal water treatment and distribution systems, isolation cubicles, and security/environmental monitoring systems, so as to meet the requirements of the Guide 1996 and the AWR 1995.

XIII.3. All proposals, plans, and construction documents which are developed in order to create new, additional animal facilities, or to renovate existing animal facilities, must be developed in compliance with the recommendations delineated in the Guide 1996, so that the program for animal care and use can be readily implemented and administered in compliance with the Guide 1996 and the AWR 1995.

XIII.4. As such, all proposals, plans, and construction documents involving the construction or renovation of space for animal care and use should be reviewed by the facility manager and the attending veterinarian to ensure that newly constructed or renovated facilities can be readily incorporated into the animal care and use program.

XIII.5. All costs of development and construction or renovation of animal facilities must be met by the host college, department, or research institution.

Appendix 1: Animal Care

1. The Manager of the Animal Facilities serves as an advocate for all animals housed at University facilities and implements and administers the Animal Care and Use Program.

2. The Manager of the Animal Facilities maintains research personnel certification files, which contain the curriculum vitae of each principal and secondary investigator, and records documenting the training and experience of all research and animal care staff members to ensure that all involved personnel have adequate knowledge and ability to perform their duties of animal care, use, and treatment.
 3. The Manager of the Animal Facilities provides management oversight of animal health and well-being, guidance and assistance with veterinary medical and surgical techniques, animal husbandry and nutrition, zoonosis control, hazard containment, and sanitation.
 4. The Manager of the Animal Facility provides supplies, and minor portable equipment, and coordinates the maintenance, repair, replacement, and certification of minor portable equipment.
 5. The Manager of the Animal Facility has management and administrative authority regarding the placement, management, husbandry, monitoring, use, and movement of animals involved in research and teaching, and regarding the use of all rooms, areas, and equipment within animal facilities on the University campuses.
 6. Although animal care, and husbandry staff may make contributions to research protocols involving animals, the PI and associated research staff named on the IACUC-approved protocol serve as the primary attending clinicians of all animals housed on behalf of that protocol. As such, research staff are responsible for providing adequate clinical oversight, and post-operative or post-procedural care of the animals, for anticipating and alleviating animal pain or discomfort whenever possible, for identifying the earliest possible clinical endpoint that contributes to the specific aims of the research, and for maintaining complete animal medical records, with entries made in sufficient detail and at intervals specified by these IACUC Policies.
 7. The Manager of Animal Facilities has authority to establish fee rates for services rendered, to invoice for, and to collect compensation from grant, contract, departmental, or other accounts for services rendered to partially meet the costs of administering the Animal Care and Use Program.
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Appendix 2: Antiserum Production (Rabbits)

1. When designing an antiserum production protocol, the applicant for animal use is directed to reviews of adjuvants and procedures of polyclonal antibody production, such as Antibodies: A Laboratory Manual, E. Harlow, & D. Lane, Cold Spring Harbor Laboratories, 1988, and those published by the National Research Council, National Academy of Science in the Institute of Laboratory Animal Research (ILAR) Journal, volume 37, number 3, pages 93-124, 1995.

2. The following is provided only as a guide in designing an immunization and phlebotomy schedule. Alternative approaches to antiserum production which involve other schedules or adjuvants, or which minimize animal use and discomfort are encouraged. Below, the use of complete and incomplete Freund's adjuvant, and the Ribi, and Titermax adjuvant systems are described.

3. Investigators should plan to adhere to one of these example protocols for antiserum production.

4. Purpose & Procedure for which animals will be used: Polyvalent antiserum, are reagents useful in immunoprecipitation, immunoblotting, enzyme-linked immunosorbent assay, and in other *in vitro* experimental procedures. Rabbits will be used to produce antiserum to antigens (typically proteins, native, polymerized, or linked to carriers). Proteins will be administered to rabbits by intradermal, subcutaneous, or intramuscular injection, blood drawn, sera clarified, the presence of antibodies which react specifically with the antigen detected and quantified, and the antiserum collected by exsanguination.

5. Characteristics of Animals: Production of antiserum is best accomplished in 2-4 kg female NZW rabbits, because they are genetically divergent from the principle sources of most antigens of interest, (i.e., human and mice), and because adequate volumes of antiserum can be readily collected.

6. Rationale of Number Requested: Antibody specificity of antiserum can vary between individual animals with respect to the dominant antigenic epitopes recognized on a given antigen, hence 2-4 rabbits are immunized with each antigen, and the resultant antiserum screened for the optimal responder.

7. Category of Research: Category C. When Freund's adjuvant is used, this can potentially produce pain, discomfort, or distress, which will be alleviated by use of appropriate anesthetics or analgesics.

8. Experimental Procedures: Standard housing and routine husbandry and handling practices will be followed. Surgery will not be performed. Substances (proteins and adjuvants) will be administered. Specimens (blood) will be collected ante mortem. No other experimental procedures will be performed.

9. Laboratory Policies: All work will be conducted within the vivarium.

10. Euthanasia: Animals will be deeply anesthetized with 44 mg/kg Ketamine and 8 mg/kg Xylazine IM, exsanguinated, a thoracotomy performed, and death assured by the absence of cardiovascular and respiratory movements.

11. Test Substances: Adjuvants and antigenic substances will be administered. When using Freund's adjuvants, complete Freund's adjuvant (CFA) will be used only for the primary immunization. Incomplete Freund's adjuvant (IFA) will be used in the second through fifth immunizations. CFA and IFA will only be administered by the subcutaneous, intraperitoneal, or intramuscular routes. Intradermal, intravenous, or footpad injections of CFA or IFA are not acceptable routes of administration. Alternative

adjuvant systems should be considered whenever possible. The Ribi adjuvant system consists of monophosphoryl lipid A, synthetic trehalose dicorynomycolate, and cell wall skeleton, and recommendations of the manufacturer are followed (Ribi Immunochem Research, Inc., 406-363-6214). The Titermax adjuvant system consists of a block copolymer CRL-8941, and recommendations of the manufacturer are followed (Hunter's Titermax 800-345-2987).

12. For all immunizations, protein antigen in saline (avoid Tris-based buffers) is mixed vigorously with an equal volume of adjuvant to generate a thick emulsion that does not disperse on the surface of saline (avoid plastic syringes).

13. Induction of an adequate immunologic response to an administered antigen, and hence the production of a useful antiserum, is influenced by the route and dose of administration. For each immunization of a protein antigen, between 50-1000 ug of antigenic protein is administered. When sufficient antigenic protein is available, >250 ug should be administered per immunization. For each injection, 10-100 ug of protein is administered per site.

14. Maximal volume of antigen-adjuvant emulsion per site, maximal number of sites of administration/animal, the gauge of needle to be used for each route are; for subcutaneous, 800 ul/site at <12 sites/animal using a 23-25 g. needle; for intradermal, 100 ul/site at <45 sites/animal using a 25 g. needle; and for intramuscular, 500 ul/site at <3 sites/animal using a 23-25 g. needle.

15. Schedule of immunizations and phlebotomies are, preimmune bleed followed by primary immunization on day 0, first immune bleed on day 14, second immunization on day 21, second immune bleed on day 35, third immunization on day 42, third immune bleed on day 56, fourth immunization on day 63, fourth immune bleed on day 77, and fifth immunization if needed on day 84, with the fifth immune bleed on day 98.

16. Animals are monitored daily, and a log of all administrations, phlebotomies, and observations are kept on animal records.

17. Specimen Collection, Ante Mortem: Blood (5-10 ml) is drawn prior to immunizations, and 12-14 days following each immunization. Animals are carefully restrained, and in some cases tranquilized with 5 mg/kg Acepromazine IM, or sedated with 44 mg/kg Ketamine and 8 mg/kg Xylazine IM for phlebotomies. Blood is drawn from the marginal ear veins using a 25 g. needle/butterfly. When an adequate titre is detected, 30-50 ml of blood is collected every 14 days as needed. Exsanguination of anesthetized animals by cardiac puncture using an 18 g. needle is accomplished when a sufficient volume of high titre antigen-specific antiserum has been collected.

18. Alternative adjuvants that may be less irritating and inflammatory, and alternative approaches to antiserum production which involve other schedules or adjuvants, or which minimize animal use and discomfort are encouraged, and must be considered. Requests for animal use to produce antiserum other than as described above are encouraged, and made by submitting a completed application for animal use to the IACUC for review.

Appendix 3: Monoclonal Antibody Ascites Production (Mice)

1. The IACUC acknowledges that the Institute of Laboratory Animal Research (ILAR) and the Office for Laboratory Animal Welfare (OLAW) have identified advantages of monoclonal antibody production in mice against which *in vitro* technologies must compete, that practical *in vitro* methods exist which can replace the ascites method, and that all requests to use the mouse ascites method need to be critically evaluated.
2. It is acknowledged that production is rapid in mice, mice can support the growth of cell lines that are often difficult to culture, the antibody in ascites is highly concentrated, and the requirements for materials, equipment, and facilities is far less. It is further acknowledged that the mouse ascites method of monoclonal antibody production can cause mice discomfort. Alternatives to ascites production of monoclonal antibodies is encouraged.
3. When critically evaluating a request to use the mouse ascites method, the applicant must demonstrate in writing to the IACUC that the proposed use is scientifically justified, that methods that avoid or minimize discomfort, distress, and pain (including *in vitro* methods) have been considered, and that the latter have been found unsuitable.
4. When considering an application for ascites production of monoclonal antibodies the IACUC considers the request in light of the following guidelines. Ascitic fluid volume will not exceed 20% of baseline body weight. Isoflurane is used as an inhalant anesthetic during abdominal paracentesis. Approximately two milliliters of warm saline or lactated ringers solution is administered SQ following paracentesis to compensate for fluid loss. The third paracentesis is performed post mortem, and only three paracentesis will be performed.
5. Animals are monitored daily and weighed regularly for the degree of abdominal distension. Regular (2-3 taps) and timely (every 1-3 days) abdominal paracentesis are required to prevent any animal discomfort (ruffled coat, hunched posture, pallor, inactivity) in advance of complications, which could evolve. Animals in discomfort are humanely euthanized. A log of all activities and observations is kept.

Appendix 4: Studies of Wild Animals In or From Natural Settings

1. The IACUC acknowledges that the federal government, including the National Science Foundation (NSF) requires its grantees to comply with the *Guide* and the *PHS Policy*.
2. The *Guide* and *PHS Policy* charge the IACUC with oversight of the experimental procedures, and methods of handling, care, and use of free-living wild vertebrate

animals. These activities must comply with the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* as outlined in Appendix D of the *Guide*.

3. As a federal grantee, UCF has filed a written assurance to comply with the *Guide* and *PHS Policy*.

4. Studies of wild animals in natural settings (referred to herein as "field studies") contribute to the conservation and well-being of wild animals. Efforts to protect indigenous animal species often are dependent on an ability to learn which species are present, the nature of their habitat, distribution, ecology, anatomy, physiology, and reproduction. The University affirms that the respectful use and treatment of all animals is both an ethical and scientific necessity.

5. The IACUC recognizes that free-living wild vertebrate animals comprise a considerable range of diversity represented by over 20,000 species of fishes, over 8,000 species of amphibians and reptiles, and over 9,000 species of birds, with varied and often poorly known behavioral, physiological and ecological characteristics.

6. The IACUC recognizes that field studies often involve many species, some of which may be unanticipated or even unknown to science before the onset of the study.

7. The IACUC recognizes that it is not always possible to predict at the initiation of field studies all potential observation or collection opportunities, the number of animals to be encountered, the species to be encountered, or the effects of research procedures.

8. The IACUC recognizes that no concise or specific compendium of approved methods for field research encompassing all species, settings, and methods is available, practical, or even desirable.

9. The IACUC recognizes that there is considerable variability among taxa of wild vertebrates in terms of their basic needs and how they should be handled, and that the PI is often an authority on the biology of the species under study, and the techniques appropriate for the conduct of the proposed study.

10. The IACUC recognizes that the number of specimens required for a field investigation will vary greatly, depending upon the questions being explored, that field studies require larger samples than laboratory studies because field have less control over biotic and abiotic conditions that produce greater variation than laboratory studies, and that a relatively large number of specimens may actually represent only a very small percentage of the population.

11. The IACUC recognizes that state and federal wildlife agencies review applications for permits for their scientific merit and their potential impact on native populations, and issue permits that authorize the taking of specified numbers of individuals, the taxa and methods allowed, the period of study, and often other restrictions designed to minimize the likelihood that an investigation will have deleterious effects.

12. The IACUC recognizes that pain perception by many species of vertebrate animals may not be uniform over the various portions of their bodies, and that broad extrapolation of pain perception across taxonomic lines may not be appropriate.

13. The IACUC recognizes that the collection of live animals and their preparation as museum specimens is necessary for research and teaching activities in systematic zoology. Each animal collected should serve as a source of information on many levels (e.g., behavior, morphology, genetics), to assure the maximum utility of each animal and to minimize the need for duplicate collecting. Formalin fixation of dead specimens is acceptable, however killing unanesthetized specimens by immersion in a formalin solution is unacceptable, unless justified for scientific reasons to the IACUC.

14. IACUC membership includes a biologist who can provide the IACUC with an understanding of the nature and impact of the proposed field investigation, the housing and care of the species to be studied, and the risks associated with maintaining wild vertebrates in captivity, per *IACUC Policy III.3*.

15. The PI must assure the IACUC in their *Application for the Study of Wild Animals In or From Natural Settings* that their field study and laboratory use of wild animals will be in accordance with the *Acceptable Field Methods in Mammalogy*, the *Guidelines for the Capture, Handling, and Care of Mammals*, the *Guidelines for Use of Fishes in Field Research*, the *Guidelines for Use of Live Amphibians and Reptiles in Field Research*, the *Guidelines to the Use of Wild Birds in Research*, *DEA regulations*, *IACUC Policies*, *PHS policy*, *AWR and the Guide*,

16. The PI assures the IACUC in their *Application for the Study of Wild Animals In or From Natural Settings* that the taxa chosen is well-suited to answer the research questions posed.

17. The PI must make an effort to understand the population status of the taxa to be studied prior to their capture or removal, and ensure that the number of animals used or removed from the wild will be the minimum necessary for accomplishing the goals of the study.

18. The PI must ensure that procedures will avoid or minimize distress to the animals consistent with sound research design.

19. Procedures that cause more than momentary or slight distress to the animals must be performed with appropriate sedation, analgesia, or anesthesia, except when scientifically justified by the PI in writing and approved by the IACUC.

20. Methods of euthanasia must be consistent with the methods recommended by the American Society of Mammalogists, American Society of Ichthyologists and Herpetologists, American Fisheries Society, American Institute of Fisheries Research Biologists, Herpetologists' League, Society for the Study of Amphibians and Reptiles, Ornithological Council, and the *2000 Report of the AVMA Panel on Euthanasia*.

21. The PI must have knowledge of all regulations pertaining to the animals under study, have obtained all permits necessary for carrying out the proposed studies prior to

their initiation. There is no need to submit copies of all permits to the IACUC, unless they are requested. The PI must ensure that studies conducted outside of the United States will also be in accordance with all wildlife regulations of the country in which the research will be performed.

22. Animals of endangered or threatened taxa must not be removed from the wild, nor imported or exported except in compliance with applicable regulations.

23. All wild animals are potentially hazardous to research staff, either from traumatic injury, infectious disease, venoms, or poisons. Staff working in the field should maintain current tetanus immunization status, and those working with carnivores or bats should maintain current rabies immunization status. The PI must ensure that the design of the field study does not compromise the health and safety of other animals in the area, or the staff working in the field.

24. The PI must assist the Animal Facility Manager with tracking its field research activities by reporting the approximate number of animals encountered or used to the IACUC.

25. The PI and associated research staff must be familiar with the animals to be studied and their response to disturbance, sensitivity to capture and restraint, and requirements for captive maintenance to the extent that these factors are known or applicable to the study.

26. The PI and associated staff must have adequate experience, training, and knowledge regarding the housing, feeding, and care requirements of the animals to be studied, to the extent that these factors are known or applicable to the study, and the PI must direct such activities in the field. The living conditions of animals held at field sites must be appropriate for the involved animals, and contribute to their health and well-being.

27. The IACUC acknowledges that although field studies in their simplest form consists of direct observation of free-ranging animals under natural conditions, the objectives of most field studies mandate that individual animals be captured one or more times. Capture techniques that have minimal impact on the animal, and are environmentally benevolent should be used whenever possible. Whenever feasible, the potential for return to the natural environment must be incorporated into the sampling design.

28. Acceptable capture techniques that have more than a minimal impact on fish include gill netting, electrofishing, the use of ichthyocides, and the use of hooks or spears; on amphibians and reptiles include trapping and netting; on birds include netting and trapping; and on mammals include trapping, netting, and capture darts which deliver an immobilizing drug. Capture devices such as nets and traps must be checked frequently to prevent animal injuries or mortality.

29. Restraint procedures of wild animals, including confinement, physical restrictions, or drug-induced immobilization must be those that cause the least amount of restraint necessary, that can be accomplished in the shortest period of time, that reduce or

eliminate contact between the handler and the animal, and that minimize hazards to personnel, whenever possible within the constraints of study design.

30. The IACUC acknowledges that the marking of wild animals is a basic method of many field studies, which provides a way of determining the movements, abundance, and population dynamics of wild animals. PIs must carefully consider the nature and duration of restraint required by the marking technique, the amount of tissue affected, whether distress is momentary or prolonged, whether the animal after marking will be at greater than normal risk, whether the animal's desirability as a mate is reduced, and whether the risk of infection or abscess formation is minimal.

31. Acceptable marking techniques of fish include fin-clipping, freeze branding, electrocauterization, tagging, radiotelemetry, or radioisotopes; of amphibians and reptiles include scale clipping, banding, tagging, shell marking, radiotelemetry, tattooing, electrocauterization, branding, or radioisotopes; of birds include banding, dyes, collars, tagging, radiotelemetry; of mammals include tagging, banding, radiotelemetry, tattooing, spot-shaving, radioisotopes, or freeze branding. The PI must consider the potential for pain and discomfort associated with each of these techniques, and whether they should be preceded by a general or local anesthetic, and/or followed by a topical antiseptic.

32. Maintenance of wild animals in their natural setting must incorporate, as far as possible, those aspects of the natural habitat deemed important to the survival and well-being of the animals. Adequacy of maintenance must be judged by monitoring factors such as appearance, activity level, general behavior, rate of growth, change in body weight, breeding success, and rate of survival. Nutritionally balanced diets must be provided, or natural foods should be duplicated as closely as possible. Natural light, ventilation, temperature, and humidity conditions should be provided, unless these are factors under investigation.

33. Methods used for sampling tissues or specimens from wild animals should be designed to obtain the maximal amount of scientific data, with the least amount of animal handling, restraint, and distress, involving a minimum number of animals. Methods that cause more than slight or momentary pain or discomfort require the use of appropriate anesthetics and/or analgesics. Aseptic sampling techniques and surgical procedures must be utilized. PIs must consider whether antimicrobial drugs should be administered following sampling or surgical procedures.

34. Whenever wild-caught animals are brought into a laboratory, they must be maintained under conditions that comply with the *Guide*, unless the purpose of the study requires the simulation of the natural setting, or when the wild animals housed in the laboratory require conditions other than those prescribed by the *Guide*. In such instances, the design of enclosures and methods of care must accommodate salient features of the animal's ecology, morphology, physiology, and behavior. PIs should consider whether newly captured animals that are brought to the laboratory be quarantined from resident animals for a period of at least 30 days.

35. Whenever practical and ecologically appropriate, as soon as possible after capture, upon completion of the study wild-caught animals should be released at the site of the

original capture, if their ability to survive has not been impaired, if they can be expected to function normally, when conditions are conducive to their survival, and when their release is not likely to spread pathogens, unless laws or regulations prohibit release, or release may be detrimental to the well being of the existing native animals.

36. All live vertebrate animal activities conducted by University faculty, students, or staff, or supported by University funds, must be proposed to, and approved by the IACUC prior to the initiation of that activity, regardless of where it will be performed.

37. The shipping and receiving of biologics, animals or plant specimens must be conducted in accordance with federal safety and importation guidelines and regulations. The PI must act in accordance with the United States Department of Agriculture, Animal and Plant Health Inspection Service regulations regarding the limits on importation of animals or biologics that may have been exposed to an exotic livestock or poultry disease agent, and the limits on the importation of plants and other vegetable matter. The PI must act in accordance with the *Public Health Service Foreign Quarantine Regulations* (42 CFR 71.54) which govern the importation and transfer of etiologic agents and vectors of human disease. The movement of other non-infectious materials such as formalin-fixed tissues, sterile cell cultures, and other preserved tissues or materials where no evidence or indication exists that they contain an infectious agent of animal or public health significance are not governed by these regulations.

Appendix 5: Some Useful Anesthetics and Analgesics - Rats & Mice

1. Comments regarding historically common anesthetics and analgesics follow:

Alpha-chloralose is useful for maintaining stable surgical anesthesia with minimal cardiovascular and respiratory depression, after induction with a more potent, short-acting anesthetic.

Atropine is an anticholinergic used to reduce bronchial and salivary secretions, and to protect the heart from vagal inhibition, which can occur during intubation, or as a result of handling of the viscera.

Acepromazine is a phenothiazine tranquilizer, produces sedation, potentiates the action of anesthetics and analgesics, and causes mild peripheral vasodilation.

Buprenorphine, Fentanyl, Meperidine, and Morphine are narcotics which produce moderate sedation and profound analgesia.

Diazepam (Valium) has both tranquilizing and sedative actions, and potentiates the actions of anesthetics and analgesics.

Droperidol is a butyrophenone tranquilizer, with similar effects as the phenothiazines (see acepromazine, above).

Ether is both flammable and combustible - and is not recommended for use without approval from various safety and biohazard committees. Ether inhalation is unpleasant and irritating, can cause profuse bronchial and salivary secretions, coughing, and laryngospasm. Ether inhalation is not an acceptable form of anesthesia within these laboratories, and can be substituted by the use of isoflurane (if an inhalant anesthetic is required for anesthesia), or by other agents, methods, or techniques of anesthesia (note: for substitutes to ether euthanasia, see euthanasia policy below).

Isoflurane is an excellent inhalant anesthetic, and should be used with a calibrated vaporizer, and with scavenging of waste gas.

Ketamine is a dissociative cataleptic, produces sedation and immobility, increased blood pressure, increased muscle tone, only slight respiratory depression, loss of the corneal blink reflex, increased salivary secretions, and variable analgesia, and can result in apnea. Surgical anesthesia can be induced when it is administered in combination with acepromazine, xylazine, or diazepam.

Neuroleptanalgesic combinations, such as Fentanyl (a synthetic opiate) and Droperidol (a butyrophenone tranquilizer), produce deep analgesia, which can be reversed by the administration of Naloxone, moderate to severe respiratory depression, poor muscle relaxation, but when administered at 50-70% of recommended dose with Diazepam induces useful surgical anesthesia in rodents and rabbits, and can be maintained by infusion of Fentanyl alone.

Pentobarbital is a barbituric acid derivative which produces surgical anesthesia, but potentially significant cardiovascular and respiratory depression, and is a perivascular irritant.

Thiopental is a barbituric acid derivative which produces smooth and rapid induction of anesthesia, but has only moderate analgesic properties, is a perivascular irritant, and can cause apnea following iv injection.

Xylazine is an alpha-2-adrenergic agonist tranquilizer, a potent sedative, has marked analgesic properties in ruminants, and potentiates the actions of most anesthetics and analgesics.

2. Mouse Doses:

PRE-ANESTHETIC MEDICATIONS

Atropine sulfate 0.02-0.05 IM, SQ, IP

SEDATIVES/TRANQUILIZERS

Acepromazine 1-2 IM, 2-5 IP

Diazepam (Valium®) 5 IP

Ketamine (Vetalar®, Ketaset®) 2-10 IM, IP

INJECTABLE ANESTHETICS

Ketamine (Vetalar®, Ketaset®) 100-200 IM, IP, 25 IV

Pentobarbital (Nembutal®) 35 IV, 60 IP

Thiopental (Pentothal®) 40-85 IP

ANESTHETIC COMBINATIONS

Ketamine and Xylazine (Note: combine 1.5 ml of 100 mg/ml Xylazine and 10 ml of 100 mg/ml Ketamine; dilute 1:4 with saline and dose at 0.1 ml/20 g b. w.). 100 & 15 IM, IP

Fentanyl & Droperidol (Innovar-Vet®) with Diazepam (Valium®) 0.2-0.5 ml/kg & 5 mg/kg IM, IP

Ketamine & Acepromazine 100 & 2.5 IM

Ketamine, Xylazine, and Acepromazine (Note: Combine 150 mg [1.5 ml] of 100 mg/ml ketamine, 30 mg [1.5 ml] of 20 mg/ml xylazine, and 5 mg [0.5 ml] of 10 mg/ml acepromazine) 0.5-0.7 ml/kg SQ, IM

INHALATION ANESTHETICS

Due to its low vapor pressure, methoxyflurane can be used without a vaporizer to anesthetize mice. Care should be taken to prevent the animal from coming into direct contact with the drug/agent. This "open" method of anesthesia must be done in a fume hood, or with some other "scavenging" system, to prevent inhalation of the anesthetic by the operator.

Other gaseous anesthetics, such as halothane or isoflurane should not be used in a closed-jar system because lethal concentrations of the gases occur at room temperature. These agents are excellent anesthetics when used with a precision vaporizer, which delivers appropriate controlled levels of anesthetic gas by mixing it with oxygen or room air.

Methoxyflurane (Metofane®) No longer available.

Fluothane (Halothane®) 1-3% (Induce with face cone) 0.5-1.5%

Isoflurane (Aerrane®, Isoflo®) up to 5% 2-3%

ANALGESICS: Also see rodent analgesic appendix 6.

Nonsteroidal anti-inflammatory drugs (NSAIDs) should be considered in place of, or as an adjunct to, opioid analgesics. Unfortunately, there is little scientific information or experience with the newest, most effective NSAIDs in animals.

Carprofen (Rimadyl®) (NSAID) Can be mixed with Jell-O.

Flunixin meglumine (Banamine®) (NSAID) Link gives dose 1.1 mg/kg IM, IV bid

Meperidine (Demerol®) (opioid) 20-60 IM, SQ

Pentazocine (Talwin®) (opioid) 10 SQ, IM, IV

Nalbuphine (Nubain®) (opioid) 4-8 IM

Butorphanol (Torbutrol®) (opioid) 0.05-5.0 SQ q 4 h

Buprenorphine (Buprenex®) (opioid) other mouse dosage programs 0.05-0.1 SQ q 12 h

NOT RECOMMENDED: Chloroform, Carbon tetrachloride, Chlorpromazine (Thorazine®), Ether, Trichloroethylene, Tribromoethanol

3. Rat Doses:

PRE-ANESTHETIC MEDICATIONS

Atropine sulfate 0.02-0.05 IM, IV, SQ

SEDATIVES/TRANQUILIZERS

Acepromazine 1-2 IM

Diazepam (Valium®) 2.5-4 IM, IP

Xylazine (Rompun®) 1-3 IM

Ketamine (Vetalar®, Ketaset®)- 2-10 IM, IP

INJECTABLE ANESTHETICS

Fentanyl & Droperidol (Innovar-Vet®) (Note: Use 10% solution) 0.2-0.4 ml/kg IP

Ketamine (Vetalar®, Ketaset®) 44-100 IM, 75 IP

Pentobarbital (Nembutal®) 30-40 IP

ANESTHETIC COMBINATIONS

Ketamine and Xylazine - (Combine 1.5 ml of 100 mg/ml Xylazine and 10 ml of 100 mg/ml Ketamine, dose at 0.1 ml/100 g b.w.) precalculated at 85 & 13 IM, IP

Xylazine (20 mg/ml) inject first or mix with Ketamine (100 mg/ml), 5-10 mg/kg, SQ 80-90 mg/kg, SQ, IP

Ketamine and Acepromazine® 75 & 2.5 IM

Ketamine, Xylazine, and Acepromazine

(Note: Combine 150 mg [1.5 ml] of 100 mg/ml ketamine, 30 mg [1.5 ml] of 20 mg/ml xylazine, and 5 mg [0.5 ml] of 10 mg/ml acepromazine) 0.5-0.7 ml/kg SQ, IM

INHALATION ANESTHETICS

Due to its low vapor pressure, methoxyflurane can be used without a vaporizer to anesthetize rats. Care should be taken to prevent the animal from coming into direct contact with the drug/agent. This "open" method of anesthesia must be done in a fume hood, or with some other "scavenging" system, to prevent inhalation of the anesthetic by the operator.

Other gaseous anesthetics, such as halothane or isoflurane should not be used in a closed-jar system because lethal concentrations of the gases occur at room temperature. These agents are excellent anesthetics when used with a precision vaporizer, which delivers appropriate controlled levels of anesthetic gas by mixing it with oxygen or room air.

Methoxyflurane (Metofane®) No longer available.

Fluothane (Halothane®) 1-3% (Induce with face cone) 0.5-1.5%

Isoflurane (Aerrane®, Isoflo®) up to 5% 1-3%

ANALGESICS: Also see rodent analgesic appendix 6.

Nonsteroidal anti-inflammatory drugs (NSAIDs) should be considered in place of, or as an adjunct to, opioid analgesics. Unfortunately, there is little scientific information or experience with the newest, most effective NSAIDs in animals.

Carprofen (Rimadyl®) (NSAID) Can be mixed with Jell-O.

Flunixin meglumine (Banamine®) (NSAID) Link gives dose 1.1 mg/kg IM, IV bid

Meperidine (Demerol®) (opioid) 10-20 IM, SQ q 2-3h

Pentazocine (Talwin®) (opioid) 10 SQ

Butorphanol (Torbutrol®, Stadol®) (opioid) 0.05-2 SQ

Buprenorphine (Buprenex®) (opioid) other rat dosage programs 0.01 - 0.05 SQ q 8-12 h

Nalbuphine (opioid) 1-2 IM

NOT APPROVED: Chlorpromazine (Thorazine®), Chloral hydrate, Chloroform, Tribromoethanol

Appendix 6: Rodent Analgesics

Over the last 10 years, our understanding of the pain response of rats to surgery and the efficacy of various analgesics has markedly improved thanks to the work from Paul Flecknell's group. From this work the following points may be useful to investigators deciding on analgesic options for their rats. The majority of studies have used laparotomy incisions as the surgical stimulus. The various authors believe this is a milder form of pain stimulus compared to other surgeries (i.e. laminectomies, crush wounds) so, although the following comments refer specifically to laparotomy pain, this represents a good starting point for formulating analgesic regimes for more invasive procedures.

1. Is vocalizing a reliable means of indicator of pain in rats? Vocalizing was never monitored in any of the studies as an indicator of pain. This supports my own impression that vocalizing (or at least that within our range of hearing) is not a useful indicator of pain in rats. This is perhaps not surprising if one considers that vocalizing is not common means of communication in this species (compared to dogs that will often vocalize pain).

2. What indicators of pain were present in these studies? General indicators of pain included: decreased water and food intake, loss of weight, and decreased locomotor activity (Flecknell and Liles, 1991; Liles and Flecknell, 1993; Liles and Flecknell, 1994; Liles et al., 1997).

More specific behavioral indicators of pain after laparotomy included: cat-like stretching, horizontal stretching (i.e. rat horizontal on the ground), withering, and twitching (Roughan and Flecknell, 2000). Behaviors that are specific to other surgical lesions are likely to exist but have not been characterized in the rat. It is important to realize that any experimental model that impairs locomotion (i.e. spinal cord injury models) may mask specific behavioral aspects of pain.

3. When should the analgesic be given? Preemptive analgesic protocols have become the preferred method of managing post-surgical pain. In support of this, administration of the analgesic prior to surgery has been demonstrated to provide superior analgesia compared to post-surgical administration in rats that received a laparotomy (Hayes and Flecknell, 1998). Analgesics should be administered prior to the surgical stimulus.

4. How much buprenorphine should be used as a single dose? The dose used in all studies for post-operative analgesia was 0.05 mg/kg (s.c.). The range 0.01-0.05 mg/kg is common in older texts and probably should be replaced with a minimum dose of 0.05 mg/kg. This is supported by a recent paper showing minimal reduction of isoflurane MAC in rats that had received 0.01 mg/kg buprenorphine (Criado et al., 2000).

5. How many doses of buprenorphine should be given? This question is difficult to answer as most studies only follow the rats during the immediate post-operative period. Additional doses of buprenorphine after laparotomy may be beneficial (Liles and Flecknell, 1993 & 1994). A second dose of buprenorphine given 9 hours after the first dose was still able to improve indicators of pain in rats that had received a laparotomy

suggesting that laparotomy pain outlasts a single dose (Liles and Flecknell, 1994). Recently it has been suggested that rats may mask pain during the dark-cycle hours to avoid displaying abnormal activity and increased risk of predation (Roughan and Flecknell, 2000). This brings up a problem in accessing presence of pain in this species during this part of their light cycle. If two doses have been demonstrated to be beneficial to rats after laparotomy, it would seem reasonable to conclude that two or more would be indicated with more extensive surgeries (i.e. orthopedic procedures, laminectomies).

6. What about NSAIDs? Considerable focus has been placed on the NSAIDs over recent years as an alternative to opioid based post-surgical pain control in veterinary species. Recently, NSAIDs have been shown to be effective analgesics in rats following surgery (Liles and Flecknell, 1994; Flecknell et al., 1999; Roughan and Flecknell, 2000). The more commonly used agents are ketoprofen (5 mg/kg s.c.) and carprofen 5 mg/kg (s.c.). These agents have the advantage of not being controlled drugs, have less respiratory and cardiovascular side effects than opioids, and are long-acting agents.

7. The combination of an opioid and NSAID for immediate post-surgical pain control has been demonstrated to be superior to an opioid alone in dogs and cats. In rats, a single combined dose of buprenorphine and carprofen has been shown to be as effective as two separate doses of buprenorphine after laparotomy (Liles and Flecknell, 1994). Anecdotally, I have use this combination in rats that receive a laminectomy and spinal cord injury and have been impressed with the speed at which these animals return to eating and drinking compared a single dose of buprenorphine.

Appendix 7: Some Useful Anthelmintics and Antibiotics

Drug	Possible Efficacy	Dosage
Droncit® , praziquantel	Tape - Taenia sp., Dipylidium sp., Echinococcus sp.	D = 5 mg/kg, C = 10 mg/kg, po, sc, im
Panacur® , febendazole	Round - Toxocara, Toxascaris sp., Strongyloides sp., Capillaria sp., Hook - Ancylostoma sp., Whip - Trichuris sp., Tape - Taenia sp.	D/C = 50 mg/kg/day, for 3 days, po
Nemex-2® , pyrantel pamoate	Round - Toxocara, Toxascaris sp., Hook - Ancylostoma sp.	D/C = 5 mg/kg, po
Milibus-V® , glycobiarsol	Whip - Trichuris sp.	D = 220 mg/kg/day, for 5 days, po
Telmintic® , mebendazole	Round - Toxocara, Toxascaris sp., Strongyloides sp., Capillaria sp., Hook - Ancylostoma sp.,	D/C = 22 mg/kg/day, for 3-5 days, po

	Whip - Trichuris sp., Tape - Taenia sp.,	
Albon® , sulfadimethoxine	Coccidia?? - Isospora sp., Toxoplasma sp., Sarcocystis sp.	D/C = 50 mg/kg day 1, then 25 mg/kg days 2-6, im, sc, po
Amprolium	Coccidia?? - Isospora sp., Toxoplasma sp., Sarcocystis sp.	D = 150 mg/kg/day, 7 days, po
Ampicillin	Broad Spectrum, Bacteriocidal; Escherichia sp., Staphylococcus sp., Clostridium sp., Shigella sp., Proteus sp., Pseudomonas sp.	D/C = 6-8 mg/kg, q8-12h, IM
Penicillin G	Broad Spectrum, Bacteriocidal; Escherichia sp., Staphylococcus sp.	D/C = 40,000 u/kg/day, IM
Cephalothin	Broad Spectrum, Bacteriocidal	D/C = 40-80 mg/kg/day, IM
Pen-Strep	Broad Spectrum, Bacteriocidal	D = 20 mg strep/kg/day, IM
Gentamycin	Broad Spectrum, Bacteriocidal; Pasteurella sp., Bordetella sp., Salmonella sp., Klebsiella sp., Pseudomonas sp., Proteus sp.	D/C = 5 mg/kg q12h day 1, then q24h, SC, IM
Neomycin	Broad Spectrum, Bacteriocidal; Salmonella sp.	D/C = 15 mg/kg q6- 24h, po
Tylosin	Narrow Spectrum, Bacteriostatic; Mycoplasma sp.	D/C = 10 mg/kg q12- 24h, IM

Appendix 8: NIH Assurance of Compliance with Public Health Service Policy on Humane Care and Use of Animals

The University of Central Florida, hereinafter referred to as the institution, hereby gives assurance that it complies with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, hereinafter referred to as PHS Policy.

I. APPLICABILITY

This assurance is applicable to all research, research training, experimentation, biological testing, and related activities, hereinafter referred to as activities, involving live, vertebrate animals supported by the Public Health Service (PHS) and conducted at

this institution, or at another institution as a consequence of the sub-granting or subcontracting of a PHS-conducted or supported activity by this institution.

“Institution” includes the University of Central Florida, the UCF Brevard Campus, the UCF Daytona Beach Campus, the UCF Downtown Academic Center, and the UCF South Orlando Center.

II. INSTITUTIONAL POLICY

A. This Institution will comply with all applicable provisions of the Animal Welfare Act and other Federal statutes and regulations relating to animals.

B. This Institution is guided by the “U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training.”

C. This Institution acknowledges and accepts responsibility for the care and use of animals involved in activities covered by this Assurance. As partial fulfillment of this responsibility, this Institution will make a reasonable effort to ensure that all individuals involved in the care and use of laboratory animals understand their individual and collective responsibilities for compliance with this assurance as well as all other applicable laws and regulations pertaining to animal care and use.

D. This Institution has established and will maintain a program for activities involving animals in accordance with the following guide:

1. Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, Washington, D.C., 1996.

III. INSTITUTIONAL PROGRAM FOR ANIMAL CARE AND USE

A. The lines of authority and responsibility for administering the program and ensuring compliance with this Policy are represented in **Appendix A**.

B. The qualifications, authority, and percent of time contributed by the veterinarian(s) who will participate in the program are stated below. Veterinary care is administered by the Attending Veterinarian. Emergency support, if needed, will be provided by two local veterinary practitioners.

Attending Veterinarian:

Norman B. Guilloud, DVM with forty years of research laboratory animal veterinary experience. Dr. Guilloud provides professional and regulatory veterinary oversight and support to the animal care and use program at the University of Central Florida (UCF). He also provides recommendations, as needed, to ensure compliance with all relevant federal, state, and local animal welfare regulations, laws, guidelines, and policies. Dr. Guilloud has direct access, as needed, to the Institutional Official. He is a voting

member of the UCF IACUC Committee. He provides animal facility management oversight as it relates to the care and welfare of animals. He also conducts both formal and informal training for animal care personnel, research principal investigators and their staff to ensure that persons handling animals being maintained at UCF are appropriately qualified and trained in the procedures outlined in animal use protocols. As attending veterinarian Dr. Guilloud provides support as needed in the planning and execution of animal research projects, including the selection of anesthetics and analgesics, and the proper pre-surgical and post-surgical and euthanasia methodologies. He serves as a consultant resource for facility construction design and planning, facility management, principal investigator experimental design as it directly relates to the welfare of research animals. Dr. Guilloud routinely visits UCF animal care facilities at least once per month. He is available and on call 24 hours per day via phone, fax, and computer email if needed for consultation or administration of medical care of research animals.

Local Emergency Veterinarians: Are available on an on-call basis for emergency purposes in the event that the attending veterinarian is not accessible.

Lawrence O. Pultz, DVM is in private practice locally and specializes in a mixed veterinary practice that includes small and large animal medicine, exotics, and birds. Dr. Pultz is equipped to provide mobile emergency veterinary care.

Woodey Dudley, DVM is in private practice locally and specializes in small animal medicine including exotics, birds, and reptiles. Dr. Dudley is a member of the Association of Avian Veterinarians. Dr. Dudley is equipped to provide mobile emergency veterinary care.

James Califf, DVM has been in private practice for 23 years. He specializes in small animal medicine and is equipped to provide mobile emergency veterinary care.

Veterinarians have the responsibility for the health care of the animals covered by this assurance. Employees responsible for the daily care are required to examine animals at least once daily, and report any signs of illness to both the attending veterinarian and the principal investigator. Health care is performed by the veterinary staff and/or the husbandry staff under the supervision of the attending veterinarian.

C. This Institution has established an Institutional Animal Care & Use Committee (IACUC), which is qualified through the experience and expertise of its members to oversee the Institution's animal program, facilities, and procedures. The IACUC consists of at least five members, and its membership meets the compositional requirements set forth in the PHS Policy at IV.A.3.b. Attached is a list of the names, degrees, position titles, specialties, and institutional affiliations of the IACUC chairperson and members.

D. The IACUC will:

1. Review at least once every six months the Institution's program for humane care and use of animals, using the Guide as a basis for evaluation. The IACUC procedures for conducting semiannual program evaluations are: at least every six months, at least two voting members of the IACUC committee will review the animal-care program, inspect the animal facilities, and activity areas. After a review and inspection, a written report signed by a majority of the IACUC will be made to the Vice President for Research.

2. Inspect at least once every six months all of the Institution's animal facilities, including satellite facilities, using the Guide as a basis of evaluation. The IACUC procedures for conducting semiannual facility inspections are: the Committee (at least two voting members) makes unannounced tours of inspection of all of the animal facilities at least every six months. Repeat visits are made to any area where concern has been expressed to ensure that recommended changes have been instituted. During these site visits, the programs for the care and use of animals are reviewed and documented. Results of these tours are recorded in the minutes and are submitted to the Vice President for Research.

3. Prepare reports of the IACUC evaluations conducted as set forth in IV.B.1. and 2. of this Policy and submit the reports to the Associate Vice President for Research and Commercialization. The reports will be updated at least once every six months upon completion of the required semiannual evaluations and will be maintained by the Institution and made available to OLAW upon request. The reports will:

a) provide a description of the nature and extent of the institution's adherence to the Guide and this Policy

b) must identify specifically any departures from the provisions of the guide and this Policy, and must state the reasons for each departure.

c) must distinguish significant deficiencies from minor deficiencies (see IV.B.3.). If program or facility deficiencies are noted, the reports must contain a reasonable and specific plan and schedule for correcting each deficiency.

The IACUC process for developing reports and submitting them to the Institutional Official is: the Animal Care Coordinator, IACUC Coordinator and/or the Veterinarian will prepare the draft report based upon the program and facility reviews. This draft report will be forwarded to the IACUC Chair or designee for review and comments. After the Chair's or designee's approval of the report, the Animal Care Coordinator and/or IACUC Coordinator will finalize the report, secure the appropriate signatures, and submit to the Vice President for Research and Commercialization.

4. Review concerns involving the care and use of animals at the institution. The IACUC procedures for reviewing concerns are: potential infractions of the Guides or of the Animal Welfare Act are investigated immediately by a designated IACUC member, who reports to the Committee for recommendations as to action. In the event that the Committee cannot be convened in time to address a situation of urgency, the Chair or designee of the Committee communicates directly with the Investigator and informs the Director of Research of any action. The Committee is authorized to suspend an activity involving animals in accord with specifications set forth in IV.C.6.

Any deficiencies brought to the Committee's attention are relayed to the responsible administrators and Principal Investigator, as appropriate. Usually two months are allowed for correction of the deficiencies or for a response as to why they cannot be corrected at that time. Any deficiencies that potentially compromise animal welfare are required to be corrected immediately.

The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present.

5. Make written recommendations to the Associate Vice President for Research and Commercialization regarding any aspect of the institution's animal program, facilities, or personnel training. The procedures for making recommendations to the Institutional Official are: the IACUC prepares semiannual reports and submits a majority signed report on a semiannual basis. If needed, additional reports can be submitted at any time by the IACUC Committee or its Chair.

Any deficiencies noted are also relayed to the responsible administrators. Usually two months are allowed for correction of minor deficiencies or for a response as to why they cannot be corrected at that time. Any deficiencies that potentially compromise animal welfare (significant deficiencies) are required to be corrected immediately.

6. Review and approve, require modifications in (to secure approval) or withhold approval of those activities related to the care and use of animals in ongoing activities as set forth in the PHS Policy at IV.C. The IACUC procedures for protocol review are: The Committee meets quarterly or more often, if needed. The presence of a simple majority of voting members constitutes a quorum. The Committee reviews protocols, discusses the results of inspections and program reviews, and conducts any other business relevant to the required functions.

No member who is personally affiliated with protocols being reviewed may participate in the IACUC review or approval of a research project except to provide information requested by the IACUC; affiliated members are asked to leave the meeting room for final discussions and voting, the affiliated member may contribute to the constitution of a

quorum but, he or she may not participate in the vote for a protocol on which they are associated or affiliated with. This is then noted on the meeting minutes.

a. Submitted protocols in which the uses of animals are indicated are evaluated by the Committee and funds are not released by the University until IACUC approval has been issued. Upon submission, the protocols are screened by the IACUC office for completeness. Completed protocols are photocopied and distributed for review to each committee member:

b. The IACUC reviews submitted protocols either at a convened meeting of a quorum (simple majority), or through the use of designated reviewers. Designated reviewers are appointed by the IACUC Coordinator. Each protocol is assigned to two reviewers in which at least one will be from the same field of study. If the protocol has a pain category of C, D or E, the protocol is assigned to at least one DVM for review.

c. The use of designated reviewers occurs only after the entire IACUC is provided with a list of the protocols to be reviewed, and each member is provided an opportunity to call for full committee review of any protocol. If a full committee review is not requested, at least one or more members of the IACUC, designated by the IACUC Coordinator and qualified to conduct the review, reviews the protocol and has the authority to approve, require modifications, or request full committee review. The IACUC Chair may also request a review by a consultant reviewer(s) who may have more expertise than any available committee member for the purpose of providing an informed, objective non-biased review of the animal use protocol. Consultants may not approve or withhold approval of an activity or vote with the IACUC unless they are also members of the IACUC.

d. In either review setting the animal user is provided with comments, questions or concerns of the committee in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval. If the IACUC decides to withhold approval of an activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or in writing to the committee. The responses are distributed to the committee or designated reviewer and a decision of approval is usually issued within 10 working days.

7. Review and approve, require modifications in (to secure approval), or withhold approval of proposed significant changes regarding the use of animals in ongoing activities as set forth in the PHS Policy at IV.C. The IACUC procedures for reviewing proposed significant changes in ongoing research projects are: Proposed significant changes of ongoing protocols are reviewed either by a convened meeting of the IACUC or by designated reviewers appointed by the IACUC Coordinator on an as needed basis. Animal users are notified prior to the expiration of their animal use approval for information regarding their intentions of continuing their previously approved activities. They are asked to reply using a form that is provided that gives the IACUC Office information regarding any changes that may have occurred or are going to occur. Based

on the information provided by the investigator the IACUC may approve or suspend an activity that it had previously approved if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, the *Guide*, the institution's assurance, or IV.C.a.-g. of the PHS Policy on Humane Care and Use of Laboratory Animals. The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present. If the IACUC suspends an activity involving animals, the Institutional Official in consultation with the IACUC shall review the reasons for suspension, take appropriate corrective action and report that action with a full explanation to OLAW.

8. Notify investigators and the Institution in writing of its decision to approve or withhold approval of those applications using animals, or of modifications required to secure IACUC approval, as set forth in the PHS Policy at IV.C.4. The IACUC procedures to notify investigators and the institution of its decisions regarding protocol review are: In either review setting (full committee or designated reviewers) the animal user is provided with comments, questions or concerns of the committee in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications to secure IACUC approval. Copies of written correspondence are maintained within files of each application in the IACUC office. If the IACUC decides to withhold approval of an activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in writing to the committee within 10 working days. The responses are distributed to the committee or designated reviewers and a decision of action is usually issued within 10 working days.

9. Conduct continuing review of each previously approved, ongoing activity covered by PHS Policy at appropriate intervals as determined by the IACUC, including complete review in accordance with the PHS Policy at IV.C.1-4. at least once every three years. The IACUC procedures for conducting continuing review are: IACUC Approvals are reviewed on an annual basis. Animal users are notified prior to the expiration of their animal use approval, and asked to state their intentions regarding continuing their previously approved activities. They are asked to reply on an IACUC form that gives the IACUC Office information regarding any changes that may have occurred or are going to occur. Based on the information provided by the investigator, the IACUC may approve or suspend an activity that it had previously approved if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, the *Guide*, the institution's assurance, or IV.C.a.-g. of the PHS Policy on Humane Care and Use of Laboratory Animals. The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present. Any protocol at the end of its third year of activity shall be rewritten and resubmitted for a full committee review.

If the IACUC suspends an activity involving animals, the Institutional Official in consultation with the IACUC shall review the reasons for suspension, take appropriate corrective action and report that action with a full explanation to OLAW.

10. Be authorized to suspend an activity involving animals as set forth in the PHS Policy at IV.C.6. The IACUC procedures for suspending an ongoing activity are:

A. Two mechanisms exist for maintaining surveillance of ongoing research. The husbandry and veterinary staff of the Institution are in excellent positions to monitor all animal projects. They are encouraged to bring any concerns to the attention of the IACUC. Secondly , protocols that appear to have a high risk of causing animal pain/distress are monitored by the IACUC, with random visits to the animals in question.

B. Ongoing protocols using animals are reviewed by the IACUC every year. Any changes in the protocol are documented. At times of inspections the IACUC takes steps to ensure that the observed use of the animals is consistent with the use approved for the project.

C. The IACUC may suspend an activity that it had previously approved if it determines that the activity is not being conducted in accordance with applicable provisions of the Animal Welfare Act, the *Guide*, the institution's assurance, or IV.C.a.-g. of the PHS Policy on Humane Care and Use of Laboratory Animals. The IACUC may suspend an activity only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present. Any protocol at the end of its third year of activity shall be rewritten and resubmitted for a full committee review. If the IACUC suspends an activity involving animals, the Institutional Official in consultation with the IACUC shall review the reasons for suspension, take appropriate corrective action and report that action with a full explanation to OLAW.

D.. The individual(s) authorized by this institution to verify the IACUC approval of those sections of applications and protocols related to the care and use of animals are Thomas P. O'Neal, PhD, Associate Vice President of Research and Commercialization & Director of Sponsored Research, Farol N. Tomson, DVM, IACUC Chair, Norman Guilloud, DVM, Attending Vet and Cristina Cammano, IACUC Coordinator.

E. The occupational health and safety program for personnel who work in laboratory animal facilities or have frequent contact with animals is:

Tetanus immunization is required for all those with substantial animal contact.

Employees are provided free access to a University Biosafety Manual and any Material Safety Sheets located in each animal facility office for their own education on University Procedures related to activities within the animal care facility.

Employees who are injured on the job receive immediate medical attention from Student Health Services or the closest Urgent Care Center outlined by the UCF HR department.

All necessary accident reports are completed and submitted in accordance with other rules, regulations, and University policy.

F. The University of Central Florida now has three animal facilities meeting the varied needs of our researchers.

Transgenic Animal Facility (TAF): The total gross number of square feet in the TAF is approximately 4000 sq.ft.. This facility is a barrier facility and maintains a strict animal health status. Animals received into this facility must meet specific health requirements and be from approved vendors or other sources as determined by the attending veterinarian. The primary species of animals housed are mice and the average daily inventory is 1600 Mice.

Biomolecular Sciences (TAF Satilite): Has multiple rooms (Room 234 and Room 332) housing animal research projects. The BMS is the building directly adjacent to the TAF and has been commissioned as of 12/01/2004 and has a room 332 being utilized as a quarantine animal room for new arrivals that may have questionable health status, and require further testing prior to allowing entry into the TAF. It also has room 234 that houses 500 mice and 70 rats in ventilated/hepafiltered caging research projects that have specialized equipment associated with the animal projects that have to be closely monitored by PI and research staff. These rooms are monitored and maintained by the TAF animal care staff on a daily & weekly basis.

Wild Animal Facility (WAF): The WAF has been designed as a conventional animal housing facility with 2000 gross square feet. This facility has been designed to house multiple species of animals under the same roof. The present daily inventory is 650 mice, and 55 rats, one reptile.

G. The training or instruction available to scientists, animal technicians, and other personnel involved in animal care, treatment or use is:

a. When evaluating protocols, the Committee pays particular attention to ensuring that the principal investigator and the support staff collectively have the required expertise to conduct the proposed experiments. Where this may be in question, the investigator is referred to an appropriate specialist on campus for advice and guidance. In addition the attending veterinarian is available to provide either formal or informal training as needed to animal use personnel if the committee feels that additional training is required.

b. An introductory "Animal Care & Use Awareness" seminar is offered to all research technicians and research faculty using animals. This seminar is offered once a year. The IACUC Office maintains records of who attends this course.

c. Specific species training sessions covering hands-on procedures are offered once a year to faculty and research technicians. These sessions concentrate on restraint, injections and blood collection. Special sessions are held on aseptic rodent surgical procedures if needed.

d. A graduate course, Laboratory Methods for Molecular Biology (MCB 6407C) is offered to graduate students in the Biological Sciences that addresses the common animal techniques and procedures used in the laboratory.

IV. INSTITUTIONAL STATUS

A. The animal facilities of the University of Central Florida are not accredited by AAALAC. The lack of accreditation does not in any way imply that substandard conditions exist. It simply means that this unit sees no significant advantage to be gained from becoming accredited by this agency at this time.

B. All the Institution's programs and facilities for activities involving animals have been evaluated by the IACUC and will be reevaluated by the IACUC at least once every six months in accordance with IV.B.1. and 2. of the PHS Policy, and reports will be prepared in accord with IV.B.3. of the PHS Policy. The IACUC has and will continue to use the Guide as a basis for evaluating the Institution's programs and facilities. Semiannual reports of the IACUC evaluations will be submitted to the Vice President for Research. Where program or facility deficiencies are noted, the individual responsible for each facility is required to correct the deficiencies noted within a designated time period. Semiannual reports of the IACUC evaluations will be maintained by this Institution and made available to OLAW upon request. The most recent semiannual report of the IACUC is attached.

V. RECORDKEEPING

A. This Institution will maintain for at least three years:

1. A copy of this assurance and any modifications thereto, as approved by PHS.
2. Minutes of IACUC meetings, including records of attendance, activities of the committee and committee deliberations.
3. Records of applications, protocols and proposed significant changes in the care and use of animals and whether IACUC approval was given or withheld.
4. Records of any IACUC reports and recommendations (including minority views) as forwarded to the Associate Vice President for Research and Commercialization.
5. Records of accrediting body determinations.

B. This Institution will maintain records that relate directly to applications, protocols, and proposed changes in ongoing activities reviewed and approved by the IACUC for the duration of the activity and for an additional three years after completion of the activity.

C. All records shall be accessible for inspection and copying by authorized OLAW or other PHS representatives at reasonable times and in a reasonable manner.

VI. REPORTING REQUIREMENTS

A. At least once every 12 months, the IACUC, through the Institutional Official, will report in writing to OLAW:

1. Any change in the status of the Institution (e.g., if the Institution becomes accredited by AAALAC or AAALAC accreditation is revoked), any change in the description of the Institution's program for animal care and use as described in this Assurance, or any changes in IACUC membership. If there are no changes to report, this Institution will provide OLAW with written notification that there are no changes.

2. Notification of the date that the IACUC conducted its semiannual evaluations of the institution's program and facilities (including satellite facilities) and submitted the evaluations to Thomas O'Neal, PhD Associate Vice President for Research & Commercialization. **The latest report is attached as Appendix C.**

B. The IACUC, through the Institutional Official, will provide OLAW promptly with a full explanation of the circumstances and actions taken with respect to:

1. Any serious or continuing noncompliance with PHS Policy.
2. Any serious deviations from the provisions of the Guide.
3. Any suspension of an activity by the IACUC.

C. Reports filed under VI.A. and VI.B. above shall include any minority views filed by members of the IACUC.