v.180901

Instructions for UHCL IACUC Protocol Form

Please fill out all information on the protocol form and attach any necessary appendices. The completed protocol should be emailed as an attachment to the IACUC Chair (with a CC: to the IACUC Coordinator) as instructed before the appropriate protocol meeting. An email will be sent to all investigators that use animal subjects informing of the protocol deadline and meeting date(s).

Throughout the entire protocol form, please answer questions in the form fields provided. The default setting is for royal blue field text. The distinct font colors will allow reviewers to easily distinguish between questions and answers on the form. Please restore the font color to blue if copying and pasting from another document. Exceptions can be made when exact formatting is important.

1. If the project is funded by a grant, the protocol title must match the grant title. This will enable UCHL to comply with any just-in-time requests for verification that the project has IACUC approval.

2. The Principal Investigator should be a faculty member or full-time staff at UHCL. The secondary contact person may be a graduate student. Use your office phone number for the primary phone, and a cell phone or home phone for the emergency phone.

3. Check "New Protocol" if this is the first time you have submitted a protocol for this project. Check "Renewal" if you had a previous protocol that has either expired or is due for an annual renewal. Check "Addendum" if you have a current protocol that needs modification. For renewals of expired protocols, provide the previous protocol number.

All protocols must be renewed each year that they are active. Check "Annual Renewal" for these protocols. After 3 years (or the length of time specified on the original protocol), the entire protocol must be reviewed. Check "de novo Review" for these protocols. You will need to fill out appropriate information in Appendix F for renewals.

Any changes to the originally approved protocol must be submitted as an addendum. Check all appropriate boxes for an addendum to indicate a "Personnel Change," "Minor Revision," or "Major Revision." You will need to fill out appropriate information in Appendix F for an addendum.

Check if this project is for 1, 2, or 3 years in length. Annual renewals will be needed for multi-year projects. After three years, de novo review application will need to be submitted if the project is to continue.

If this protocol is for the use of animals in a class, check "Instruction" and indicate the course name and number (e.g., PSYC 5235, Learning Principles), and how frequently the course is offered (e.g., every Fall and Spring semester, once every two years).

If this protocol is for the use of animals in research, indicate the funding mechanism for the research.

4. If you plan to house animals in the UHCL Animal Research Facility (ARF), please consult with the Director of the ARF to make sure there is adequate space for your research project. Housing of animals in other areas for more than 12 hours at a time will require inspection and approval of those areas by the IACUC as meeting guidelines for proper animal housing and welfare. Additionally, a standard operating procedure will need to be submitted in Appendix G describing how animal welfare will be maintained. This does not apply if animals will be housed at other animal facilities that have AAALAC accreditation (e.g., UH, NASA).

5. The lay summary requested is important for explaining the value of research using animals. It should be written so that non-scientific members of the IACUC can understand without difficulty. Scientist members of the committee unfamiliar with your research also rely on this section to understand the goals of the project. Furthermore, this statement may be provided to representatives of regulatory agencies concerned with animal use or released in response to requests made under open records laws.

The lay summary should be written so that it clearly describes the goals and potential benefits to be derived from the work. All approved research or training using animals must be of benefit to humans and/or animals. This assertion is obvious for clinical or applied studies, but it also holds for studies considered basic research. The summary should be as succinct as possible and should use terms non-scientists can understand. When writing this lay summary: avoid technical jargon; explain any technical terms you must use; keep words, sentences, and paragraphs short; use examples; explain how you arrived at your conclusions. The lay summary should include the following information: the goals and intended benefits of the project; the species and number of animals to be used (if applicable); how animals will be used.

6. Indicate the animals that are planned to be used, their source, and numbers that will be used each year. Place each species or strain on a separate line. The number of animals should match the answer to number 9. The following is an example of how this section can be filled out:

Species (common name)	Breed/Strain	Sex	Vender/Source	Number Requested			
				Year 1	Year 2	Year 3	Total
Rat	Sprague Dawley	F	Harlan	20	20	20	60
Rat	Long Evans	F	Harlan	15	15	15	45
Mouse	C57/BL	F	Jackson Labs	0	20	20	40

Field Studies: Choose one of two options

1. In some field studies, it is not feasible to know exactly which species will be encountered. Check the Not Applicable box if this applies to your project.

2. If the project plans to study/survey specific species, check all boxes that apply on the second line.

7. List the experience and qualifications of the PI and all personnel who will be supporting this protocol along with their role in each procedure listed. Only personnel listed on the protocol are approved to work with animals, and only for the procedures indicated in the protocol. All personnel who will perform animal anesthesia, surgery, or other experimental manipulations must be qualified through training or experience to accomplish these tasks in a humane and scientifically acceptable manner.

The following is an example of how to complete this section:

Name, Degree, Title	Species and Years of Experience	Specific Role in Project[1]	CITI Training Date	Occupational Health Training Date

[1] Examples include: supervision, care/handling, anesthesia, surgery, monitoring, post-procedural care, euthanasia in the stated species

Jane Smith, PhD, Asst Prof John Brown, BS, grad Res Asst	Rats - 15 Rats - .5	Supervision, care/handling, monitoring, euthanasia in rats Care/handling, monitoring in rats	9/15/17 9/20/17	9/15/7 9/20/17

☒ Student visitors will/may participate in this protocol and will be supervised by: Dr. Smith

Personnel listed by name are faculty, staff, students, or outside personnel that will either work on the project for longer than one semester or will be involved in a significant role (e.g., performing surgery, euthanasia) in the project, often without direct supervision.

A student visitor is a person who works or observes in a laboratory on a temporary basis (no longer than one semester) and does not work with animals without direct supervision. Faculty investigators or vendors who are visiting UHCL to demonstrate techniques or equipment are exempt from this requirement.

A student visitor may, with direct supervision, handle or perform minor procedures on animals. A student visitor will not be allowed to perform survival surgery, even under supervision. A student visitor does not need to be included on an IACUC protocol as "Research Personnel" or added to the protocol by an amendment; however, he/she must complete the minimum training requirements outlined below. If the student visitor becomes a permanent member of the laboratory staff or continues to work in the laboratory longer than one semester, he/she must be added to the protocol via an Amendment to Add Personnel.

It is the responsibility of the Principal Investigator to ensure that the student visitor receives training in proper animal handling and welfare along with training in specific techniques used. The student visitor must also fill out the Occupational Health Program forms.

8a. A literature search is to be performed using at least two different databases. Examples of databases are PubMed (Medline), PsycInfo, Agricola, Biological Abstracts, Google Scholar, and the Animal Welfare Information Center (remember that PubMed contains the same database information as Medline). The date the literature search was conducted should be indicated, along with range of years covered by the databases. Keywords included in the search should be listed. These should include the species and any potentially painful/stressful procedure in order to determine if any alternative procedures exist. Additionally, you must insure that your research does not unnecessarily duplicate previous research.

For each of the following sections, keep in mind the "3R's," Replacement, Reduction, and Refinement. Replacement is replacing animals with non-animal techniques such a computer models or *in vitro* assays. Reduction is reducing the number of animals used. Refinement is changing procedures to reduce pain or distress in animals.

8b. Provide the rationale and purpose of the proposed projects along with its significance. While this does not have to be a long statement, you should build a case based on previous research or scientific need that indicates that the experiments planned is justified. This would include information typically seen in the introduction of a research article.

8c. Justify why animals must be used for this project. This should address the concept of "Replacement." Why can non-animal models such as isolated organ preparation, cell or tissue culture, or computer simulation not be used in the experiment? Convenience, cost, or tradition is not a sufficient justification.

8d. Why is the particular species requested most appropriate for this project? Could another species of animal, such as an invertebrate, be used instead? Convenience, cost, or tradition is not a sufficient justification.

8e. Are alternative procedures available that would cause less pain or distress in animals? This should address the concept of "Refinement." Animals listed in USDA Category B and C by definition do not experience any pain or distress (see number 10).

8f. According to federal law, "The principal investigator has provided written assurance that the activities do not unnecessarily duplicate previous experiments." Check the box if you agree with the statement. If not, provide a justification why replication is necessary. Acceptance of new ideas in science is often dependent upon the ability of other scientists to duplicate published reports. The IACUC can allow duplication of previous work if you can convince them that it is important scientifically to do so.

9. Justify the number of animals requested in the proposal. This should address the concept of "Reduction." The minimum number of animals to produce scientifically valid and reliable results should be used. Provide the number of animals in each group along with the total number of animals. A list or table of groups and sample sizes could be helpful. A rationale should be given as to why numbers were chosen. If a power analysis was conducted, details such as values used for effect size, power, and alpha should be included. If prior research is used as a justification, please provide citations.

Some field studies may not be able to give exact number of animals that are planned to be surveyed. In these cases, a justification of the sampling method should be included.

10a. Check the USDA Pain/Distress category that indicates the highest level of pain/distress that animals will experience during the proposed experiments. Even if 90% of animals used will experience no pain/distress, while 10% will experience pain/distress, you must check the category corresponding with the 10% that will experience pain/distress. Use the following descriptions to determine the category your study falls into. If you do not know which category your research should be classified as, the IACUC can be consulted. (Note: there is no Category A)

Category B
Animals being "bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes." These animals have not been used for any research procedure, however minor. Category B is the place to put breeders and other animals that are not undergoing any experimental procedures. Non-invasive observation only of animals in the wild.

<u>Examples</u>

- Standard aquaria husbandry procedures not for research, teaching or testing.
- Standard animal health programs, e.g., routine physical examinations & vaccinations, performed by experienced professionals.
- Normal maintenance of non-wild sourced fish.

Category C

Animals that are not subjected to procedures that involve pain or distress or would require the use of pain-relieving drugs. Routine procedures such as injections and blood sampling from veins that produce only mild, transient pain or discomfort are reported in this category. Another example of category C procedures is an observational study of animal behavior. Animals that are euthanized before tissue collection or other manipulations are also commonly placed in this category, if no other procedures performed that put them in a higher pain/distress category.

Examples

- Normal maintenance, breeding, conditioning, or holding of animals for use in teaching or research, or use of animals in teaching where no category D or E procedures are involved (e.g., therapeutic riding, pet grooming).
- Teaching routine physical examinations/performance, or routine physical examinations by students.
- Manual restraint of awake animals to perform routine examinations, or the time necessary to complete any category C procedure.
- Holding or weighing animals.
- Injections, blood collection, or catheter implantation, via superficial vessels.
- Behavioral testing without stress.
- Feeding or oral/gastric gavaging studies.
- Collection of tissues preceded by standard euthanasia.
- Chemical immobilization/ restraint for ≤ 60 minutes (e.g., use of MS-222, clove oil or medetomidine in fish or amphibians).
- Tagging fish without surgical procedures.
- Ear punching, tail clipping, or toe clipping of laboratory or captive animals. (Note: If animals must be captured/ trapped first, the animal use should be categorized as D.)

Category D

Animals subjected to potentially painful procedures for which anesthetics, analgesics, or tranquilizers will be used. The important concept is that animals are given appropriate anesthesia and/or pain relief to limit their pain and distress as much as possible.

Examples

- Induction of behavioral stress.
- Non-survival surgical procedures.
- Cannulation.
- Survival surgery with anesthesia and without significant post-operative pain management (e.g., biopsy).
- Implantation of minor chronic catheters (e.g., femoral arterial and venous catheters).
- Short-term food or water deprivation (≤ 24 hours).
- Capturing/trapping of live animals (e.g., collecting fish using commercial fishing practices, or trapping wild birds, rodents, or amphibians).

- Tagging studies involving surgical procedures.
- Perfusion under anesthesia.
- Use of chemical or immunological adjuvants (e.g., ascites production, Freund's adjuvant).
- Physical restraint of awake animals (> 15 minutes).
- Inducement of a functional deficit.
- Chronic maintenance of animals with a disease/functional deficit.

Note: Dissection after euthanasia is not considered surgery.

Category E
Animals are subjected to painful or stressful procedures without the use of anesthetics, analgesics, or tranquilizers. Withholding of anesthetics, analgesics, or tranquilizers can only be allowed if it is scientifically justified in writing and approved by the IACUC.

Examples

- Research or procedures that require continuation until death occurs (e.g., fisheries mortality studies).
- Application of noxious chemicals or stimuli if animals cannot avoid/ escape the stimuli and/ or it is severe enough to cause pain or distress.
- Continuous withholding of food or water (> 24 hours) from birds or mammals.
- Use of electric shocks or other methods of stress which would cause pain or distress in humans.

10b. Must be filled out if Category E is selected. Reasons why drugs or other methods to alleviate pain or distress will not be used must be clearly stated. Convenience or cost is not a valid justification. Indicate the number or percentage of total animals that will be in this category. Category E studies are given increased scrutiny by the IACUC because they must be satisfied that less painful or stressful alternatives are not available, or that less painful/stressful endpoints cannot reasonably be used. By law, the institution must annually report all category E procedures to the USDA and include a scientific justification supporting the IACUC's decision to approve them. Often, the justification given by the researcher on the animal forms submitted to the IACUC is used for the annual report.

It is important for information on category E procedures to be complete and accurate. Once submitted to the USDA, this information will likely be available to the public through a Freedom of Information Act request.

11. Animals that are place for adoption cannot have undergone an evasive experimental procedure. An adoption form must be filled out by the PI and person adopting the animal. If possible, the PI should consider transferring animals to other protocols. This facilitates the reduction in the total number of animals used for research. If the animal is to be euthanized, identify the AVMA Guidelines on Euthanasia accepted method to be used. If a deviation from these methods is required, a justification is needed in box 11b.

12. Attach any appropriate forms to the protocol form. If an appendix is not needed, delete the pages from the protocol. Do not submit with blank pages or "N/A."

13. Check each box to acknowledge your acceptance of each statement. You do not need to sign the electronic copy of the protocol, but a hard copy with a signature will be required.

Appendix A: Laboratory Research or Classroom

A1. A scientific justification must be given for deviations from normal animal care routines. Convenience or cost is not a justification.

A2. Provide a complete description of the proposed use of the animals. This description should allow the IACUC to understand the course of an animal from its arrival through the experiment to the endpoint of the study, and final disposition. A flow chart can be helpful in describing the sequence of experimental events. Explain the procedures performed with the animals, when they will occur, and the number of animals used. Please include:

- The approximate time period the animals will be on study
- Description of animal identification methods
- Radiation (dosage and schedule)
- Use of restraint devices
- Sites, volume, and frequency of collections of fluids and tissues
- Name/type, dosages, and routes of administration of all compounds and other materials administered
- Animal manipulations (such as injections, behavioral, breeding, etc.)
- Procedures for behavioral studies
- Summary of surgical manipulations (Details of surgery will be placed in Appendix B)
- Details of field research will be placed in Appendix C

A3a. Procedures that produce pain or distress in humans are expected to produce the same effects in other vertebrates. How will pain or distress be minimized in animals? This can include more than pain relieving drugs, such as behavioral strategies such as habituation, or reducing time of exposure.

A3b. A list of common criteria for determining pain or distress in animals to determine endpoints is listed. If a deviation is needed, a justification should be provided (e.g., the experimental manipulation should cause weight loss).

Appendix B: Surgical Procedures

Answer all questions asked. Delete this page if the protocol does not involve surgical procedures.

Appendix C: Wild Animal and/or Field Research

Answer all questions asked. Delete this page if the protocol does not involve wild animals and/or Field Research.

Appendix D: Non-Pharmaceutical Grade Drugs

Answer question 1. If the proposed drug is not listed in question 1, answer questions 2 and 3 in their entirety. Delete this page if the protocol does not involve non-pharmaceutical grade drugs.

Appendix E

Answer all questions asked. Consultation with other units at UHCL to ensure safety may be required before IACUC approval.

Appendix F

1. Check if this is a renewal or addendum and what type of renewal or addendum. Multiple boxes can be checked (e.g., an annual renewal with minor revisions). If no changes are made in the renewal, check "No Changes" in addition to type of renewal.

2. Check the current status of the project.

3. List any changes to research personnel. The research personnel section on the original protocol should be updated as well.

4. If there are any methodological changes, briefly describe the changes so that it is easy for a reviewer to find modification to the protocol. The original protocol should be modified to accurately represent the current methodology utilized in the project. New text should be indicated with underline. Using different color text or highlighting may not reproduce well in black and white.

If there are no changes in methods, leave the original protocol as is with Appendix E as an attachment.

5. Answer the question.

6. Answer the question. You do not need to attach the full text of journal articles, published abstracts, or presentations. Citations are sufficient.

Supplemental Information

The following are the guidelines for IACUC protocols.

Guide for the Care and Use of Laboratory Animals

Protocol Review

The animal use protocol is a detailed description of the proposed use of laboratory animals. The following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC:

- rationale and purpose of the proposed use of animals
- a clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee
- availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation [2]
- justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis[3])
- unnecessary duplication of experiments
- nonstandard housing and husbandry requirements
- impact of the proposed procedures on the animals' well-being
- appropriate sedation, analgesia, and anesthesia (indices of pain or invasiveness might aid in the preparation and review of protocols[4])
- conduct of surgical procedures, including multiple operative procedures
- postprocedural care and observation (e.g., inclusion of post-treatment or postsurgical animal assessment forms)
- description and rationale for anticipated or selected endpoints
- criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated
- method of euthanasia or disposition of animals, including planning for care of long-lived species after study completion

---

[2] National Research Council 2011. Guide for the Care and Use of Laboratory Animals. Appendix A, Alternatives. Available online at https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf.

[3] National Research Council 2011. Guide for the Care and Use of Laboratory Animals. Appendix A, Experimental Design and Statistics. Available online at https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf.

[4] National Research Council 2011. Guide for the Care and Use of Laboratory Animals. Appendix A, Anesthesia, Pain, and Surgery. Available online at https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf.

- adequacy of training and experience of personnel in the procedures used, and roles and responsibilities of the personnel involved
- use of hazardous materials and provision of a safe working environment.

v.180901

Federal Regulations:

TITLE 9--ANIMALS AND ANIMAL PRODUCTS
CHAPTER I--ANIMAL AND PLANT HEALTH INSPECTION SERVICE, DEPARTMENT OF AGRICULTURE
PART 2_REGULATIONS--Table of Contents
Subpart C_Research Facilities
Sec. 2.31 Institutional Animal Care and Use Committee (IACUC).

(Sections describing the membership of the IACUC omitted)

(d) IACUC review of activities involving animals.

(1) In order to approve proposed activities or proposed significant changes in ongoing activities, the IACUC shall conduct a review of those components of the activities related to the care and use of animals and determine that the proposed activities are in accordance with this subchapter unless acceptable justification for a departure is presented in writing; Provided, however, That field studies as defined in part 1 of this subchapter are exempt from this requirement. Further, the IACUC shall determine that the proposed activities or significant changes in ongoing activities meet the following requirements:

(i) Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals;

(ii) The principal investigator has considered alternatives to

procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, e. g., the Animal Welfare Information Center, used to determine that alternatives were not available;

(iii) The principal investigator has provided written assurance that the activities do not unnecessarily duplicate previous experiments;

(iv) Procedures that may cause more than momentary or slight pain or distress to the animals will:

(A) Be performed with appropriate sedatives, analgesics or anesthetics, unless withholding such agents is justified for scientific reasons, in writing, by the principal investigator and will continue for only the necessary period of time;

(B) Involve, in their planning, consultation with the attending veterinarian or his or her designee;

(C) Not include the use of paralytics without anesthesia;

(v) Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure or, if appropriate, during the procedure;

(vi) The animals' living conditions will be appropriate for their species in accordance with part 3 of this subchapter, and contribute to their health and comfort. The housing, feeding, and nonmedical care of the animals will be directed by the attending veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied;

(vii) Medical care for animals will be available and provided as necessary by a qualified veterinarian;

(viii) Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures;

(ix) Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and

aseptic techniques. Major operative procedures on non-rodents will be conducted only in facilities intended for that purpose which shall be operated and maintained under aseptic conditions. Non-major operative procedures and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic procedures. Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures;

(x) No animal will be used in more than one major operative procedure from which it is allowed to recover, unless:

(A) Justified for scientific reasons by the principal investigator, in writing;

(B) Required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian; or

(C) In other special circumstances as determined by the Administrator on an individual basis. Written requests and supporting data should be sent to the Animal and Plant Health Inspection Service, Animal Care, 4700 River Road, Unit 84, Riverdale, Maryland 20737-1234;

(xi) Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, Sec. 1.1 of this subchapter, unless a deviation is justified for scientific reasons, in writing, by the investigator.

(Sections describing how and how often the IACUC should review proposal omitted)

(e) A proposal to conduct an activity involving animals, or to make a significant change in an ongoing activity involving animals, must contain the following:

(1) Identification of the species and the approximate number of animals to be used;

(2) A rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used;

(3) A complete description of the proposed use of the animals;

(4) A description of procedures designed to assure that discomfort and pain to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research, including provision for the use of analgesic, anesthetic, and tranquilizing drugs where indicated and appropriate to minimize discomfort and pain to animals; and

(5) A description of any euthanasia method to be used.