INSTRUCTIONS FOR COMPLETING
THE ANIMAL CARE AND USE PROTOCOL (ACUP)

Contents

A. General Instructions

In order to facilitate the submission and approval of your animal research protocol, it will be helpful for you to understand the objectives, viewpoint, and requirements of the Animal Welfare Committee (IACUC). Prior to submitting proposals for Committee approval, new investigators need to receive an orientation about the ethical issues of animal research and IACUC operating procedures from the IACUC Chairperson. In addition, an orientation to the Central Research Facilities (CRF) and the Animal Care and Use Program provided by the Manager of the CRF is required prior to the approval/implementation of animal research protocols. You may arrange for these orientations by contacting the Manager of the CRF (444-5788).

The Animal Welfare Committee is committed to the continued use of animals in research within the constraints of humane treatment. Most investigators show similar concern for the well-being of animals. However, evidence of this concern is often not documented in the submitted protocols. In order to justify approval of procedures the committee must have adequate written information regarding the steps you will take to assure humane treatment of your animals.

1) You must justify the use and number of any animals to be used.

2) State clearly and honestly any potential discomfort you expect the animals to experience. The members of the committee may be unfamiliar with your experimental procedures, but are all well-acquainted with animal studies. If a procedure seems likely to produce discomfort and you fail to explicitly deal with this issue, the committee may require more information before final approval.

3) To help us evaluate the discomfort experienced by animals in your protocol, you may compare your procedures with human experiences during similar diseases or clinical procedures; use standard veterinary and/or human criteria for evaluating well-being, etc. See the Guidelines for Pain/Distress Classifications.

4) Not all experiments can be done without some discomfort. If yours is such an experiment, you must document that:

- alternative experiments that would produce no discomfort are unsatisfactory
- you will take steps to assure that you will cause no more discomfort than necessary
- the duration of the discomfort will be as brief as possible

- you have appropriate plans to monitor and correct problems (e.g., by use of analgesics or euthanasia).

5) Be certain that your answers in Appendix 4 (management of substances known to be hazardous) are in compliance with the Institution's standard practice instructions and/or standard operating procedures for the use of hazardous materials.

6) Although it is not the primary function of this committee to evaluate scientific objectives, scientific issues are considered. For example, if you are repeating published studies, point out the how the new information or methods justify that this is necessary.

7) **Present your material clearly, concisely, and legibly. Do not leave any blanks - use N/A (not applicable) if appropriate. The application should be understandable to the lay members of the committee**. Members of the committee donate their time -- they are more likely to act favorably the first time if applications are easy to read!

8). A Research Plan is required to provide scientific justification for the proposed studies. Attach the grant application that has been submitted for external funding or prepare a research plan using the format described below.

Organize the plan to answer these questions: (A) What do you intend to do? (B) Why is the work important? (C) What has already been done? (D) How are you going to do the work?

Include sufficient information in the plan to facilitate an effective review without reference to any previous application. Be specific and informative and avoid redundancies. Reviewers often consider brevity and clarity in presentation as indicative of a principal investigator/program director's approach to a research objective.

When a revised application is submitted, provide a statement specifying what significant changes have been made. Include additions, deletions, revisions, and any responses to criticisms in the previous summary statement.

The suggested format for a research plan is as follows:

Specific Aims. State concisely and realistically what the research described in this application is intended to accomplish and/or what hypothesis is to be tested.

Significance. Briefly sketch the background to present proposal, critically evaluate existing knowledge, and specifically identify the gaps which the project is intended to fill. State concisely the importance of the research described in this application by relating the specific aims to longer-term objectives.

Progress Report/Preliminary Studies. New applications may use this section to provide an account of the principal investigator/program director's preliminary studies pertinent to the application, and/or any other information that will help to establish the experience

and competence of the investigator to pursue the proposed project. Continuation applications would use this section to list the accomplishments on the research project during the reporting period. The titles and complete references to appropriate publications and completed manuscripts may be listed. Supplementary background graphs, diagrams, tables, and charts relevant to the progress report/preliminary studies may also be submitted.

Experimental Design and Methods. Discuss in detail the experimental design and the procedures to be used to accomplish the specific aims of the project. Describe the protocols to be used and the tentative sequence or timetable of the investigation. Include the means by which the data will be analyzed and interpreted. Describe any new methodology and its advantage over existing methodologies. Discuss the potential difficulties and limitations of the proposed procedures and alternative approaches to achieve the aims. Point out any procedures, situations, or materials that may be hazardous to personnel and the precautions to be exercised.

B. Guidelines for Pain/Distress Classification

The USDA regulations require that "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 . . . used to determine that alternatives were not available" [9 CFR Part 2.31(8)].

You will be required to provide this narrative in the Search for Alternatives section of the form. To help you more accurately complete this section, the USDA's classifications of pain/distress are detailed below.

The following guidelines are offered by the Animal Welfare Committee (IACUC) to assist research investigators in making appropriate pain/distress categorizations when writing animal care and use protocols. The IACUC has the prerogative to review and possibly alter any pain/distress categorization contained in the protocol submission.

CATEGORY:

Category C. Minimal, Transient or No Pain and/or Distress.

These procedures are considered to produce minimal, transient or no pain and/or distress when performed on small and/or docile animals by competent individuals using recognized methods.

1. Administration of:

    a. Anesthetics, analgesics, and tranquilizers
    b. Fluid and electrolyte therapy
    c. Immunizations, when not expected to cause more than minimal morbidity or distress.

d. Oral medications.

2. Non-chronic catheterization.

3. Blood collection, except transcardial and periorbital procedures in species without an orbital (retrobulbar) sinus.

4. Gastric lavage.

5. Certain procedures performed in the normal practice of veterinary medicine or animal care (e.g. injections, palpations, skin scraping, ear punching, etc.).

6. Hybridoma/ascites production, when the volume of fluid does not produce respiratory distress and paracentesis is performed only once.

7. Euthanasia as performed in accordance with the recommendations of the current AVMA Guidelines for Euthanasia. Euthanasia of fetuses and neonates needs to be performed under anesthesia utilizing either hypothermia (in rodents) or anesthetic drugs (in larger animals). Fetuses collected after the death of the dam are assumed to be nonviable.

Any of the above procedures may be painful and/or distressful to a large and/or infrequently handled and difficult to manage animal; these procedures should be listed in Category D or E. For example, even simple procedures done with nonhuman primates are placed in Category D since anesthesia is needed to avoid undue anxiety and resultant aggression. Similarly, such procedures for swine (without using specialized restraint equipment) generally require anesthesia or sedation to prevent anxiety and uncontrolled movements, and are considered as Category D.

Category D. Pain or Distress Relieved by Appropriate Measures.

Examples of procedures that may produce pain or distress, but which are performed using appropriate and adequate anesthesia, analgesia, or tranquilization and are followed with appropriate measures to alleviate pain or distress are as follows:

1. All forms of bleeding in which the animal is exsanguinated; orbital bleeding; and intracardial blood collection.

2. All surgical procedures, including those associated with “acute” experiments performed under anesthesia from which the animal does not recover.

3. Injections of agents which cause morbidity or induce excessive inflammation or necrosis. The amount of pain or distress that results from the injection(s) of an irritating or noxious agent will depend on the agent used, the site of injection and volume injected, and the animal species. Animal users should use their own judgment when classifying their particular procedures. Base the judgment on observation of the animal(s) and extrapolation of the causes of pain and distress in man.

4. Skin or corneal corrosivity testing.

5. Procedures requiring prolonged restraint of any animal (greater than 12 hours).

6. Polyclonal antibody production with immunization via intradermal injection (or via other routes if an adjuvant is used) where there are visible or clinical signs of inflammation.

7. Intracranial inoculations in animals with a fully ossified cranium.

Category E. Unrelieved Pain or Distress

Procedures listed in Category D above must be listed in Category E if they are performed without appropriate and adequate anesthesia, analgesia, or tranquilizers **or** if they are not amenable to relief by therapeutic measures. This would include assays that result in significant mortality, procedures resulting in moderate to severe morbidity, footpad inoculations, and the administration of drugs or irradiation which may cause toxicity.

C. Common Abbreviations and Definitions

ABBREVIATIONS

ACUP - Animal Care and Use Protocol

AWC - Animal Welfare Committee (IACUC)

AWEC - Animal Welfare Executive Committee

IACUC - Institutional Animal Care and Use Committee

DEFINITIONS

Activation - Formal notification from the Grants and Contracts Office of Research Administration to the researcher authorizing initiation of research project activities. Required for projects that will use external funds to support the work, such as grants or contracts.

Applications -Institutionally standardized forms which must be completed and submitted by the researcher to the IACUC.

Approval - Formal notification from the Research Protection Office of scientific approval of the research protocol by the IACUC. Approval does not constitute authority to begin research activities. (see Activation)

Continuing Review Progress Report - Written report of current project activities submitted by the researcher to the IACUC for review and approval for continuation of project

Continuing Approval- IACUC approval to continue research project activities (required at least yearly). Formal notification authorizing continuation of research activities, is sent from the Research Protection Office to the researcher.

Research Project - Systematic gathering and analysis of information in order to contribute to general knowledge. The overall design of the project is generally set forth in a written protocol.

Research Protocol - The formal design or plan of an experiment or research activity.

Surgery - Any procedure that results in the invasion of any body cavity, or joint space or produces permanent impairment of any kind.

- **Acute Surgery** - The animal is euthanized at the end of the surgical procedure before recovering from anesthesia.

- **Survival Surgery** - Surgery performed on a live animal under general anesthesia, from which the animal is expected to recover.

D. Form Specific Instructions

INSTRUCTIONS FOR PREPARING
LIFESPAN IACUC ANIMAL CARE AND USE PROTOCOL (ACUP)

- The ACUP form must be typed. Do not leave any blanks. Answer all questions. Use N/A if appropriate.
- Complete and include only the Appendices that are applicable to this project.
- The ACUP form is available at www.Lifespan.org/research/.
- **You must attach a copy of the external funding (grant) application, if applicable, or submit a separate research plan along with the ACUP.**
- **21 complete copies are required for review**. Please use double sided copies whenever possible to conserve paper.
- **You must attach copies of the lab privileges and training forms, as well as health surveillance receipts for each individual included on the personnel list**

Please contact Roland Lariviere (phone 444-2093, email rlariviere@lifespan.org) if you require assistance.

Section Heading	***Most Common Issues/Errors***
Face Page	• Contact dates, Be sure to contact the Vet and CRF Manager for every application, including *de novo* submissions • Funding issues, if you do not provide a cost center to pay for animal expenses, then you will not be able to start the project • If this application is part of a grant application and you do not have a cost center, then you cannot start the work until the grant is funded, or until you provide a departmental (unrestricted funds) cost center to pay for the animal charges. • Departmental signatures from the Dept. Chief (not Division head) are required.
1. Nature and Purpose 1a. Technical abstract	Use the outline below when preparing the structured technical abstract. • Background: Present the ideas and reasoning behind the proposed work. • Objective/Hypothesis: State the objective/hypothesis to be tested. Provide evidence or rationale that supports the objective/hypothesis. • Specific Aims: State the specific aims of the study. • Study Design: Briefly describe the study design including appropriate controls.
1. Nature and Purpose 1b. Lay summary	• Simplify language, imagine that you are describing your work to a high school student or a reporter for the local newspaper • Define technical terms. Most of the people who read the application will not have experience in your field • Be sure to explain what you are trying to do (testable hypothesis) and why animals are needed to address the aims of the project
2. Procedures	• Be sure to include Insufficient details about any procedure

	that will be done on animals • Define endpoints • Specify how often are animals are checked, especially after invasive, painful, or distressful procedures?
2. Procedures 2b. timeline	• Provide an outline or flow chart describing how animals will be used from the time when they arrive in the facility until euthanasia
2. Procedures 2c. substances	• List all substances and agents that will be administered to animals • Be sure to include the route, does and frequency of administration
3. Personnel	• Collaborators, list all personnel at this institution and from other organizations that will play a key role in this work
4. Description of animals 4b. Table	• Include USDA categories for all animals. • If an animal will be subject to more than one type of activity, such as breeding (cat B) and surgery (cat D) , then only list the highest USDA pain category (cat D) • Be sure that the number of animals described in procedures description, justification for the number of animals, and all appendices match the number listed in the table • Include breeders in category B
4c. justification for species	• List references for the use of the model • Explain why you have selected this species for the specific procedure(s)
4d. justification for numbers of animals requested	• This section is one of the hardest for researchers to complete. You must demonstrate to the reviewers how you determined the number of animals that you need. • Be sure to specify the number of groups to be used and the number of animals per group as well as the method

	used to determine group size • It may be helpful to create a table to clarify complex designs/breakdown #s by group • Define the statistics/power analysis that were used to determine group size • Delete any references to cost • Be sure that the number included in this section match the number of animals requested in 4b
4e. source	• Separate by species and/or strain • List all sources including commercial suppliers and non-commercial (ex. colleagues at other institutions, breeding colonies, rederivation, etc)
4f-m housing and diet	• Specify the max # to be housed at any one time so that the animal facility can plan for your housing needs • Completely describe how and where animals will be transported. Include the use of covered cages and non-public corridors/elevators/stairs • Description of caging
5. List of procedure types	• Be sure to answer all questions
6 Euthanasia	• Verify doses of anesthesia with Veterinary Staff
6e criteria	• Define endpoints
6f. contingency plan	• Define specific signs of pain and distress • Specify frequency of monitoring • The plan must cover all animals from the time of receipt
7. Search for Alternatives	• Two distinct databases must be searched • Keywords must relate to the painful aspects of the procedure, must include the species, and years searched, must extend for at least 15 years. For example, if you are searching for alternatives to perfusion, you must include

	the laparotomy incision, perfusion, alternative and the species in the keywords. • Do not include procedures that are not painful, or involve only momentary pain (such as injections and tail snips)
Assurance	• Two distinct databases must be searched • Keywords must relate to scientific model • This is a separate search from the search for alternatives. Keywords related to alternatives are not appropriate for this search • Years searched must extend for at least 15 years
Appendix 1 Surgical Procedures Section A.	• Specify the number of animals to be used for each procedure • Describe skin prep for incisions • Verify anesthesia regimen with Veterinary staff • Describe any monitoring that will be done to assess pain and distress, include the time intervals when animals will be checked • Do not include momentary procedures such as injections in the list of painful procedures
Appendix 1, Section B, Survival Surgical Procedures	• If you will not provide post-op analgesia then you must provide a scientific justification • When justifying why analgesics are being withheld, be sure to include a list of literature references to support any claims that you make that analgesics would interfere with the model

	• Specify how incisions will be closed • Include specific signs of pain/distress to be monitored • Specify pre-op fasting if applicable
Appendix 1B Non-Surgical but Painful or distressful Procedures Section A.	• Specify the number of animals to be used for each procedure • Describe any monitoring that will be done to assess pain and distress, include the time intervals when animals will be checked
Appendix 2 Tissue Harvest	• Include tail snips in the list of tissue to be collected (if applicable) • Specify the amount of tissue/fluid to be collected and the frequency for harvest • Specify and anesthesia/analgesia used during tissue harvest
Appendix 4 Hazards	• Do you have approval from the Biohazards and Lab Safety Committee? • If using viral vectors or other recombinant DNA technology, have you obtained approval from the Rec. DNA Committee? • Describe how you will contain the hazardous agents. • Include the route and dose for agents that are administered to live animals

Appendix 5 Breeding	• Include topical anesthetic for tail snips done in rodents older than 1 week • Clearly define the number of animals to be bred and the number of these animals that will be used for your experiments. • Clearly describe the breeding scheme **Remember**: you will be required to submit monthly reports of the animals born and used