

## *Criteria for Protocol Completion & Criteria for Protocol Review*

- *To be used by both Principal Investigators when completing an application and by PACUC members when reviewing applications.*
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**General Statement:** A well written protocol should be a complete, stand-alone document (i.e., it should never refer to another protocol) that has sufficient detail to allow for a thorough review and understanding of the protocol. The protocol should be detailed and explained in such a way that the reviewer reasonably understands what is happening to the animals before and after procedures are performed. All abbreviations/acronyms should be defined.

The first step in reviewing a PACUC protocol is having a good understanding of what to look for in order to ensure that deficiencies are prevented and ensuring an effective animal care and use program at Purdue University. To complete a thorough protocol review, the following must occur:

A protocol **must** address the three “Rs” – **reduction, replacement, and refinement** (Guide pp. 4-5).

- I. Reduction – strategies that reduce the number of animals used.
  - a. Limiting group size to the minimum needed to obtain statistically significant data
  - b. Performing multiple experiments simultaneously so the same control group can be used for all experiments.
  - c. Sharing tissues with other investigators, if appropriate
  - d. Designing experiments so animals serve as their own controls
  - e. Using newer instrumentation that improves precision and reduces animals needed per data point.
- II. Replacement – replacing the use of animals with non-animal techniques.
  - a. Use of cell culture techniques to replace animals as incubators for cell lines
  - b. Use of immunologic bench assays to replace bioassays involving animals
  - c. Use of computer software to model the pharmacokinetics of drugs in place of animal studies
- III. Refinement – designing/changing experiments or procedures to reduce pain or distress in those animals that must be used (does the treatment regime cause undue pain or distress to the animals).
  - a. Ensure that appropriate anesthesia, sedation, and analgesia is allotted to animals when necessary
    - i. New anesthetics, sedatives, and analgesics that provide reduced stress and faster recovery
    - ii. Description of drug or agent using
    - iii. Dosage using and frequency given (“as needed or as indicated for analgesic” is not an appropriate response)
    - iv. How drug/agent will be administered

- v. How is post-op care and monitoring provided
    1. Who is providing post-op care?
    2. How long will animals be monitored post-procedure and how often?
    3. Will arrangements be made for afterhours care?
  - b. Modification of research procedures to be less invasive, painful or stressful to subjects
  - c. Modification of animals' habitat for environmental enrichment and normal behavior
- IV. Search for Alternatives (USDA Policy 12,) must include
- a. A rationale for using animals
  - b. The appropriateness of the species and the number of animals to be used
  - c. A description of procedures to assure that discomfort or pain will be limited
  - d. A description of drugs to be used to minimize pain
  - e. Narrative of sources used to consider alternatives (two databases must be used)
    - i. literature, conference materials and/or database searches
  - f. Narrative that activities do not unnecessarily duplicate previous experiments
  - g. Keywords used for search include the species to be used (i.e., mice), the term "alternatives," and any potentially painful procedure(s) to be performed.

References: <http://ntp.niehs.nih.gov/pubhealth/evalatm/index.html>;  
<http://ntp.niehs.nih.gov/pubhealth/evalatm/iccvam/index.html>

**The following criteria require a protocol be sent to the Full Committee of PACUC for review:**

- **Death as an Endpoint.**
- **Pain Category E.**
- **No analgesia being provided post-surgery/procedure.**
- **Multiple Survival Surgery (if both surgeries are considered major).**
- **Use of greater than 10,000 animals on a protocol (mammalian species only).**
- **Other (i.e., Exceptions to the Guide, protocol that a committee member marks FCR, etc.).**

**What to look for when writing/reviewing a PACUC protocol**

▪ **Protocol Details**

**A. General Info – (PACUC Administrative Staff Review)**

- Fields that must be completed include:
  - Protocol Type
  - Project Type
  - Title
  - Application Date

**B. Investigator/Study Personnel – (PACUC Administrative Staff Review)**

- All Study Personnel must be listed in this tab (this tab must match the personnel information in section 2.1 of the protocol application attachment).

**C. Species/Groups – (PACUC Administrative Staff Review & PACUC Member Review)**

- Fields that must be completed include:
  - **Study/Teaching Group (i.e., should separate out by study groups with the total per study group)**
  - Species
  - [Pain Category](#)
  - Count Type
  - Count (this number should match the justification provided in section 1.3 of the protocol application attachment).
  - Exception (if applicable)

**D. Alternative Search – (PACUC Administrative Staff Review & PACUC Member Review)**

- Two (2) databases must be searched for each protocol submission (new or triennial).
  - The terms "alternatives," the species name (e.g., dog, cat, chinchilla), and any potentially painful procedure (e.g., tumor inoculation, blast injury) must be included as key words used in the literature search.
  - If "Other" is selected as a database, the name(s) of the database(s) must be indicated in the Comment box.
  - If submitting an Amendment adding a species and/or potentially painful procedure, a new alternative search must be completed using the appropriate keywords.

- **Questionnaires**

**Protocol Addendum – (PACUC Member Review)**

- Fields that must be completed include:
  - 1.1 Justification for choice of species/animal
    - The species selected should be the best suited to the goals of the research or teaching activity.
    - Acceptable Justification could be based on :
      - Species specific genetic characteristics or reagent availability
      - Required size
      - Building upon existing data base
      - Unique biological characteristics
  - 1.3 PI should Supply 1-2 references to support justification for using animals
  - 1.4 All applicable vendors should be listed
  - 1.5 Select all Special Considerations (i.e., Survival Surgery, Multiple Survival Surgeries, Food or Fluid Restriction, Prolonged Restraint, etc.)
  - 2.1 Will animals be kept for 12 hours or more outside the housing facility?
  - 2.5 Need to know if the animals will be housed in socially compatible groups?
    - Social housing is the default method of housing unless otherwise justified based on:
      - social incompatibility (i.e., inappropriate behavior)
      - Scientific necessity (must be approved by PACUC).

- Veterinary Concerns
- Pregnant females
- For short term recovery post-operatively
- Following standard agricultural husbandry practices
- 2.7 Need to know if environmental enrichment will be provided to the animals on this project.
  - Environmental enrichment should be provided unless there is a research or teaching related justification for why it is not provided
- 2.8 PI needs to specify what environmental enrichment will be provided.
  - Environmental enrichment should be adapted to each species
- 2.10-2.12 PI needs to specify if endangered, wild species, permits are needed
  - Issued by the U.S. Fish & Wildlife Service
  - Department of Natural Resources
- 3.1 Need to know who would be an appropriate person listed on this protocol, if other than the PI, to contact regarding training.
- 3.2 Need to know the name of anyone other than the professional animal care staff who will be performing husbandry.
- 3.3 PI should identify the individual(s) responsible for providing daily animal husbandry.
  - Most animal areas have animal care staff, however the PI may have special needs that may need to be handled only by a designee of his/her choosing.
- 3.4 Need to know if Laboratory Animal Program (LAP) veterinarians will be providing the primary veterinary care for the animals described in the protocol.
  - LAP Vets should be the default unless the PI has other approved vets providing primary care
- 3.8 Need to know where complete medical records will be kept and readily available to LAP veterinary staff and outside inspectors (for examples/template, contact LAP).
- (4.1-4.9 must be thoroughly reviewed by the Biosafety Officer and/or Animal Exposure Occupational Health Specialist. Other DMR's are welcome.) –
- 4.1 It is the Principal Investigator's responsibility to inform all personnel, including animal care staff, of potential hazards and contact REM.
- 4.2 The PI should review the [REM](#) webpage for safety requirements for their specific type lab. Based on this review, the PI should indicate if any additional training is needed for their staff.
- 4.3 All persons handling animals should be made aware of the Animal Exposure Occupational Health Program.
- 4.5 PI should know and indicate what precautions will be taken to protect personnel involved in the protocol (personal protective equipment).
- 4.6 If hazardous agents are used, a description of the hazardous agent(s) should include: dose, route, and frequency for treatment, post-mortem handling of animals or tissue, and any other exposure scenario for personnel.

- 4.7 If there are any special waste and animal disposal requirements (carcinogen, biohazard, radiation, etc.) a description should be given and how it will be handled.
- 4.8 If animals will be contaminated/radioactive, a description of how animal care personnel will be informed of how to handle animals and their bedding and cages should be provided.
- 4.9 The Chemical Hygiene Plan must be reviewed by the PI and with each employee and graduate student.
- 5.1-5.9 Certifications – all must be checked “yes.”

### **Triennial Renewal Supplement – (PACUC Member Review)**

The Triennial Renewal Supplement questionnaire must be fully completed for all Triennial Renewal submissions. CoeusLite Questionnaires are dynamic and will only present questions that are required based on preceding answers. All questions in the Protocol Addendum questionnaire should be completed or revised for Triennial Renewals.

- Fields that must be completed include:
  - [R.1] Are there any experiments in this renewal application that were also described in your currently approved protocol?
  - [R.3] Are there any previously approved animals that have not been utilized yet?
  - [R.5] Provide a brief summary of your progress to date (research and testing projects only).

### **Annual Continuation – (PACUC Member Review)**

The IACUC Annual Continuation questionnaire must be fully completed for all Annual Continuation submissions. CoeusLite Questionnaires are dynamic and will only present questions that are required based on preceding answers.

- Fields that must be completed include:
  - [C.1] Do you want to deactivate this protocol?
  - [C.2] During the past year, have you made any changes in the personnel listed on your protocol?
  - [C.3] If personnel have been added, and they will be performing tasks on this protocol, have they been added to the protocol via the submission of a CoeusLite IACUC Amendment? --- (IMPORTANT: If they have not been added via an Amendment, please complete one indicating their specific involvement in animal care and use activities. New personnel may not be added to the protocol via Annual Continuation submissions.)
  - [C.4] Identify any personnel who are listed on this protocol but who are no longer involved with the project.
  - [C.5] For each species listed on your protocol, indicate the number of animals that were used during the past year.
  - [C.6] Does this protocol cover an academic or teaching course?

- [C.8] Considering the health of your animals, please describe any serious (resulting in death or affecting more than one animal) or chronic problems (recurring problems or problems having a duration of one week or more) that were encountered during the past year? If not applicable, enter "NONE".
  - [C.9] Considering the approved procedures you conducted using animals during the past year, did you encounter any unexpected difficulties or complications that had an adverse impact on the health or well-being of your animals?
  - [C.11] Do you have any comments, questions, or concerns that you would like to bring to the attention of the PACUC?
- **Protocol Attachment – (PACUC Member Review)**
    - A. **Justification for Animal Use and Species**
      - 1.1 PI needs to address why alternatives (e.g., less painful/distressful animal procedures, use of phylogenetically lower species or non-animal procedures) could not be substituted (i.e., why live animals must be used)?
        - Note: ANY POTENTIALLY PAINFUL/DISTRESSFUL PROCEDURES IN THIS PROJECT MUST BE ADDED AS A KEYWORD USED IN THE LITERATURE SEARCH in the Alternative Search Tab.
        - Must take into account the 3 Rs
        - Should consider other possible animal or non-animal models
        - Should take into account research/teaching objectives and endpoints
      - 1.2 PI should state in terminology that can be understood by someone with minimal knowledge of the specific scientific area the objective(s), including the rationale for using vertebrate animals.
      - 1.3 PI should indicate the scientific rationale for the number of animals to be used including how they determined the number of animals required. **Stating that an analysis was done is not sufficient to scientifically justify the number of animals. A thorough explanation of the power analysis or method used to determine the number of animals per group should be provided. This may include the desired level of power (e.g., 80%) and effect size from similar previous experiments.** Included in the explanation should be
        - The numbers per group, number of groups, power analysis used, number of animals needed for training, pilot studies, etc.
      - 1.3b(a,b,c): The PI should provide information on how their breeding colony will be maintained.
      - 1.4 PI should indicate where the animals will be housed and the animal Procedure Area(s), and Surgery areas.
        - 1.4a: PI should indicate any special housing/cage changing/husbandry needed.
      - 1.5 If aquatic species are being used on this protocol, how the water quality parameters will be monitored and how frequently will the monitoring be done should be clearly stated.
      - 1.6 If genetically modified animals (GMA's) are being generated, how will the new lines generated be monitored for conditions that could negatively affect the well-being of the animals?

- 1.7 If animals on this protocol are considered critical or irreplaceable, how does the PI plan to preserve the line of this irreplaceable animal (e.g., cryopreservation)?
- 1.8 If fluid and/or food will be restricted to any animals on this protocol (not for surgery purposes), then a detail description of the amount and duration of such restriction must be stated.
- 1.9 If pharmaceutical grade drugs or chemicals are used for this protocol, then the following should be answered:
  - 1.9a Justification for using the non-pharmaceutical grade drug or compound (e.g., necessary to meet scientific goals, need to replicate methodology, inappropriate concentration or formulation of available pharmaceutical grade, pharmaceutical grade vehicle not appropriate for planned route of administration, non-availability of pharmaceutical grade. Note: cost savings alone is not an adequate justification) should be provided
  - 1.9a2 A description of the steps that will be taken during the preparation of the drug or compound to ensure sterility (e.g., use of filter, sterile diluents, sterile container if appropriate), the appropriate pH as feasible and that an appropriate non-toxic vehicle/diluents will be used. Include proposed shelf life/use by date, labeling method to include drug or compound name, date prepared, expiration date and storage method.
  - 1.9a3 A description of **who** will be responsible for monitoring the drug or compound preparation and use and any relevant experience handling the drug or compound.
  - 1.9a4 A description of the site and route of drug or compound administration to include potential side effects and adverse reactions and who will be responsible for monitoring the animals and how they have been trained to look for adverse reactions.
  - 2.0 If expired medical materials (see PACUC Policy at: [www.purdue.edu/animals](http://www.purdue.edu/animals)) such as fluids, sutures, catheters, implants, etc. will be used in any procedures, to include acute terminal procedures the type of expired material to be used, the type of procedure for which it will be used (acute/terminal vs. survival), and the justification of the usage should be describe. Also how such materials will be clearly labeled and segregated from non-expired materials should be indicated.

**B. Personnel Training Qualifications – (PACUC Administrative Staff Review)**

- The names of all individuals (including the PI as project director) who will be conducting the procedures on animals should be listed along with qualification numbers and specific procedure personnel will be responsible for.

**C. Surgical Procedures – (PACUC Member Review & Specifically, LAP Veterinary Staff)**

- 3.1 If there will be surgical procedures on this protocol items 3.2-3.15, should be completed if not section 4 should be completed.

- 3.2 The training the surgeon has received to perform the surgical procedures listed on the protocol application should be listed.
- 3.3 The description of the preoperative procedures that will be performed to prepare the animal(s) for surgery (e.g., fasting of animal(s) to include length of fast, withholding of water to include length of time withheld, pre-anesthetic, analgesic or antibiotic administration [include dose in mg/kg and route of administration], catheter placement or other procedures) should be described.
- 3.4 If surgery will be done aseptically
  - 3.4a describe aseptic procedures (e.g., include information regarding how instrument/ equipment is sterilized; how animal is prepared such as hair clipping and skin disinfection and use of drapes; and surgeon preparation such as hand scrub, use of sterile gloves, sterile gown, cap, mask).
  - 3.4b If no, an explanation should be provided. NOTE: Terminal surgeries greater than 6 hours should be performed using aseptic technique.
- 3.5 A detailed description of any survival and/or non-survival surgical procedures should be provided such that the PACUC reviewers can determine what procedures are actually being performed.
- 3.6 The anesthetic drugs to be used during the surgical procedures should be specified to include dosage(s) [in mg/kg of body weight or percent concentration of gases] and route(s) of administration.
- 3.7 The name(s) of veterinarian(s) or other sources consulted in regard to use of drugs should be listed.
- 3.8 PI needs to address how it will be determine that the animal is adequately anesthetized throughout the procedure.
- 3.9 If muscle relaxants or paralytic drugs will be used:
  - 3.9a A detailed description detailing the monitoring name(s), dosage(s), and route(s) of administration of these drugs along with the justification for the use of these drugs should be provided.
- 3.10 A description of any physical methods used to support the animal during surgery (e.g., circulating warm water heating pad, electrical heating pad, blankets, fluid administration, etc.) should be given.
- 3.11 If the surgery will involve recovery from anesthesia then:
  - 3.11a A description of post-surgical care and monitoring to include any physical methods used to support animal such as heating blanket and fluid administration should be provided. It should also include frequency of post -procedure observations, how long observation will continue and individual(s) responsible for monitoring animal in immediate postoperative period (until animal can ambulate) and thereafter including after-hours, weekends, and holidays as applicable.
  - 3.11b PI should indicate when sutures or staples will be removed from the animal?
- 3.12 If postoperative analgesics will be provided to relieve pain in animals:



- 3.12a PI need to provide the agent used, dose (mg/kg), route, frequency of administration (e.g., times per day), and duration of administration (e.g., days). NOTE: “As needed” is not an adequate response. PI should also indicate if the animals will be checked and what signs will be used to determine the need for analgesic administration?
- 3.12b If no, a justification for not using postoperative analgesics need to be provided.
- 3.13 PI needs to state whether post-operative complications can reasonably be anticipated. How potential complications can be detected, managed, and resolved and the specific criteria that will be used to decide when to perform euthanasia prior to completion of the study or to otherwise relieve the suffering (e.g., refusal to eat, loss of body weight, tumor ulceration or total burden, health problems refractory to medical intervention, etc.).
- 3.14 A planned record keeping (pre-surgical, surgical, and post-surgical) should be described to include the location at which such records will be maintained that will allow access to LAP, USDA, and PACUC as needed (for both survival and non-survival).
- 3.15 If more than one major survival surgical procedure will be performed on any one animal then:
  - 3.15a A detailed justification on the scientific necessity for performing more than one procedure on an individual animal should be given as well as a description of the interval between the surgeries (Cost may not be used as scientific justification).

**D. Non-Surgical Procedures – (PACUC Member Review: Specifically Scientist & LAP Veterinary Review)**

- 4.1 A general non-surgical use of animals, including pilot studies, should be described in this section using terms that can be understood by those not familiar with the area of expertise.
- 4.2 A clear, concise, sequential description (i.e., timeline) of the specific experimental design involving the use of animals that is easily understood should be included in this section.
- 4.3 A description of the procedures to be performed on the animals including drugs and chemicals should be given (e.g., blood collection = sq, diet restriction, anesthesia = ketamine/xylazine, euthanasia = cervical dislocation).
- 4.4 A description of how the procedures performed *could* have an impact on the animals’ health and well-being should be included.

**E. Anesthesia/Analgesia/Pain Relief for Non-Surgical Studies – (LAP Veterinary Review)**

- 5.1 The PI needs to state whether the animals will be subjected to any non-surgical procedure that might cause more than momentary or slight pain or distress (see list below). 5.1a. If yes, each painful/distressful condition must be checked.

- 5.2 The approximate period of time animals may experience such pain or distress should be indicated.
- 5.3 If anesthetics, analgesics, and / or tranquilizers will be used then the agent(s) used, dose (mg/kg), route, frequency of administration (e.g., times per day) and duration of administration (e.g. days) should be provided.
  - 5.3a. NOTE: “As needed” is not an appropriate response for this section unless accompanied by a description of the signs that will be used to determine the need for anesthetic, analgesic, and/or tranquilizer administration.
- 5.4 The name(s) of veterinarian(s) or other sources consulted in regard to use of drugs should be listed.
- 5.5 The PI needs to explain how they can determine whether an animal is adequately anesthetized throughout the non-surgical procedure.
- 5.6 Precaution to protect personnel should be stated if anesthetic gases are used
- 5.7 The non-surgical post-anesthetic care and monitoring should be described
- 5.8 A justification should be provided if any animals will undergo non-surgical procedures in which pain or stress is not relieved with the use of anesthetics, analgesics, tranquilizers or euthanasia

**F. Humane Endpoints – (PACUC Member Review & LAP Veterinary Review)**

- 6.1 The specific criteria that will be used to decide when to perform euthanasia prior to completion of the study or to otherwise relieve the suffering must be listed. See website for assistance <https://www.purdue.edu/research/regulatory-affairs/animal-research/policies-guidelines.php> Under Guidelines, “Humane Endpoints for Research and Teaching Animals.”
  - 6.1a The PI needs to address what specific training was provided to personnel responsible for assessment and recognition of the humane endpoint listed above.

**G. Animal Disposition – (LAP Veterinary Review)**

- 7.1 If the animal(s) will be euthanatized, then 7.2-7.5 should be completed. If not, then section 7.6 should be completed.
- 7.2 A brief description of the method of euthanasia including, agents, doses, and routes of administration and who will euthanize the animals should be stated.
- 7.3 Method of chemical euthanasia that will be used to assure that the animals will not recover should be listed. For a list of methods see website <http://www.avma.org/resources/euthanasia.pdf>
- 7.4 If you are using a physical method of euthanasia (e.g., cervical dislocation, decapitation), justify the need for use of this method.
- 7.5 How carcasses are to be disposed must be checked if applicable
- 7.6 Live animal disposition must be checked if applicable:

**• Amendments**

General Comment: Amendments should be thoroughly written/reviewed and the relevance to the originally approved protocol established. When reviewing an amendment, the reviewer should acquaint themselves with the original protocol to ensure that the current amendment is relevant to the study and/or teaching activity.

- Items that must be addressed in an amendment:
  - Investigators/Study Personnel: If adding personnel, did the PI provide the responsibilities that the person would be performing on the protocol so that the new person's qualifications can be reviewed to ensure they are indeed qualified to perform such procedures?
  - Species/Groups: If the PI added a new species, did he/she re-run the literature search to include this new species? If the PI is requesting additional animals be added to the protocol, was sufficient justification provided for this request?
  - Procedures: Did the PI provide a list of procedures the amendment would cover (i.e., blood collection, second survival surgery, additional anesthesia, etc.)?
  - Details/Explanation: Did the PI provide adequate details as to what is being added/changed with the submitted amendment?

Understanding what to look for when reviewing a protocol is the first step in preventing deficiencies and ensuring an effective animal care and use program at Purdue University. If while reading a protocol, all issues relating to the 3R's are not addressed or important fields missing, ask the Principal Investigator (PI) for clarification. If clarification still does not provide the necessary answers you are seeking, or if you believe that there is undo harm of distress to the animals, please send to full committee for review.

#### **References:**

[USDA Regulations](#)

[PHS Policy on Humane Care and Use of Laboratory Animals](#)

[AAALAC International Connection \(Winter/Spring 2001\)](#)

[Guide for Care and Use of Laboratory Animals, 8<sup>th</sup> edition](#)

[NIH Office of Extramural Research \(Frequently Asked Questions\)](#)