IACUC Protocol Form Training Manual – IACUC Reviewers

1. Protocol Title

Is a title present?

Progress Report for Renewing Protocols: Is this section complete, if necessary?

2. PI Contact Record

Is this section complete?

3. Emergency Contact

Is this section complete?

4. General Information

Compare the narrative, especially the Lay Description, of the Protocol with these check boxes.

Teaching Project: This box should be checked if the project is directly related to an undergraduate or graduate course only. It should not be checked if it is a research project that has participation by students.

Collaborative Project: Will any animal use described in the Protocol will be conducted at a different institution? Are any of the participating personnel not part of the PI staff? If this box is checked, the Lay Description must include a clear description of which experiments are occurring at each location and who will participate at the external location(s).

GLP Study: Is the Protocol being conducted under GLP conditions?

Rodent Breeding: Is rat or mouse breeding described in the protocol? Has a Breeding Addendum been completed?

Hazardous Materials: Is a hazard being used in the protocol? Hazards include:

(i) All microorganisms, including viral vectors, cancer or other cell lines, development of new transgenic animal lines (not use or breeding of existing lines), recombinant DNA including PCR (Institutional Biosafety Committee approval)
(ii) Toxins, hazardous chemicals, including isoflurane (Risk Management and Safety approval)
(iii) Radioactive or radiolabeled compounds, UV, X-Rays, MRI, Cobalt 60 (Radiation Control approval).

Has a Hazardous Material Addendum been completed? Are all the hazards identified (isoflurane and MRI are often missed as hazards)? Is the appropriate approval included as part of the Protocol?

5. Participating Personnel, Protocol Contacts & Occupational Safety Designee (OSD)

Is there more than just the PI listed on the protocol? Is it possible that other personnel will likely participate on the protocol and they have not been listed? Is an OSD listed?

6. Funding Source

Note the funding source. A funding source must be identified for all protocols. For federally funded (e.g., NIH, NSF, USDA) projects the animal portion of the grant must be available for a side-by-side review. In this case, the grant needs to be compared to the protocol to determine whether the drugs, procedures, animal numbers, etc… are congruent between the two.

7. Documentation

Check the dates and key words for both searches. The dates need to be within that last 3 months.

The keywords for the scientific literature search must be able to identify any duplication of the proposed research. The keywords for the "alternative" database must include the keywords alternatives, pain, distress, euthanasia, analgesia, reduction, refinement as well as keywords that could identify whether any alternative to the proposed animal research is available (either not using animals or using less painful/invasive methods).

Make sure there is a summary of findings for both searches. There should be some type of narrative, not just a list of references.

8. Objective/Hypothesis

This should be written in non-technical language and should provide the rationale for performing the proposed study. Any non-standard acronyms should be spelled out and technical jargon should be explained. Note whether the PI indicates if this is a pilot project.

Specifically, this section should include:

i. Why the study is important/of value
ii. What the overall goal (objective) of this research is and/or a brief description of what the research is addressing
iii. The specific aims of the study (hypothesis), and how they will be achieved using animals

If necessary, remind the PI of the difference between the information in Section 8 and 9.

9. Lay Description of Procedures

Ensure that the PI has spelled out exactly what will happen to the animals from the time they enter the facility until euthanasia. Compare the Lay Description with the Category of Manipulation and the species and animal numbers requested. Especially compare the listed procedures, including the euthanasia method, with the checked boxes in Section 12.

10. Animal Usage

a. Category of manipulation: Make sure that the Category of Manipulation is congruent with the procedures being performed. The Category of manipulation is determined by the most invasive procedure on an individual animal. Common mistakes to look for include:

<u>Using the wrong Category of Manipulation.</u>

Category A is only appropriate for field observation (no animal handling), breeding colonies with no tissue sampling for genotyping, a special diet/water that will result in no clinical symptoms or euthanasia prior to tissue harvest. If the animal is subject to any procedure prior to euthanasia for tissue harvest, this is not Category A, e.g., is restrained or trapped, is bled, is tail-tipped, receives an injection or any other type of drug administration, undergoes behavioral analysis, or is subject to diet/water restriction.

Category B is used for procedures causing only short term pain or distress. These include bleeding, tail-tipping, injection, any type of drug administration, behavioral analysis, environmental manipulation, live capture and release, non-painful restraint (by the definition in the Restraint section), standard farm animal husbandry and/or diet/water restriction.

Category C is used for, surgery or other procedures performed while the animal is anesthetized and the animal will be euthanized without regaining consciousness. This category is used for non-survival surgery.

Category D is used for surgery, tumor development, infection, physical trauma and/or other painful procedures that will be alleviated with palliative therapy. If the animal is placed in Category D then there must be a description of the palliative therapy provided and it must be appropriate to the level of pain that could be experienced. Category D is also used for procedures where an animal will receive hazardous material (biological, chemical or radiation), prolonged restraint, diet/water restriction for more than 12 hours or severe environmental stress. Please note that anesthesia for non-painful procedures such imaging is also Category D.

Category E is used for surgery, tumor development, infection, physical trauma and/or other painful procedures (described in Category D) that will not be alleviated with palliative therapy. Category E procedures must be well justified. Also note, that it may be possible to alleviate pain associated with some of the procedures (see the Example under Section 12). PIs must justify why palliative therapy is withheld for all procedures in Category E animals.

Listing an individual animal multiple times based on Category of Manipulation. The animal should always be placed in the Category of the most painful procedure it will receive.

Example

Experimental Group	Species	Category of manipulation	No. Animals	Max. days
Field observation	Amphibians (Frogs, toads, salamanders; all species)	A	500	1 day
Field observation	Lizards (Gila monster, Chuckwalla)	A	300	1 day
Field observation	Snakes (all *Crotalus* species)	A	150	1 day
Paint marking	Snake (Western diamondback)	B	20	1 day
Blood collection	Gila monster	B	10	1 day
Skin swab	Chuckwalla	B	20	1 day

In the Example above, the 10 Category B Gila monsters (blood collection) should not be included in the Category A Gila monsters (field observation). Similarly, the 20 Category B snakes should not included in the Category A snakes.

Example
20 rats are received. All are ear notched (Category B). 10 of these are subjected to tissue harvest (Category A). The remaining 10 are subjected to survival surgery with palliative therapy (Category D).

In this case, 10 rats are Category B and 10 rats are Category D

Common mistakes include listing the animals as: 20 in Category B and 10 in Category D
10 in Category A and 10 in Category D
20 in Category B and 10 in Category A and 10 in Category D

Ensure that the total number of animals in each Category adds up to the total number of animals requested.

b. Justification for Category of Manipulation and Species: Ensure that the Justification for Category of Manipulation is appropriate to the procedures and Categories listed.

Examples
Animals in Category A will under go field observation only and will not be handled. Snakes and lizards placed in Category B must be trapped before marking, blood collection or skin swabbing (all Category B activities).

Rats placed in Category B will undergo ear notching (identification technique) prior to tissue harvest. Rats placed in Category D will be subjected to surgery X with drug Y for palliative therapy following ear notching.

Make sure that a justification as to why a "lower species" cannot be used is included.

c. Statistical Justification: This section results in many requests for additional information by the IACUC. There are three straightforward ways to provide statistical justification of the animal numbers requested. Depending on the complexity of the work, more than one statistical justification may be required.

i. Use of a statistical package/formula. Include the name of the package/formula, and the *p* value being used to determine significance.
ii. Reference to a similar study (the PIs or others) using similar animal numbers/group sizes, where a statistically meaningful result was obtained. Make sure the full reference is cited.
iii. The PIs unpublished data on similar studies using similar animal numbers/group sizes, where a statistically meaningful result was obtained. The use of "in my experience" as the sole justification is not sufficient. Instead, the PI should provide a brief overview of the study and the data and whether statistical significance was achieved. However, the PI should use a published study where possible.

Somewhere in the Protocol (usually here or in the Lay Description) should be statements that the animal numbers include controls and what provision for failure rates are included.

11. Housing

Make sure that in addition to the building, room numbers are included. Compare whether any rooms are listed as for anesthesia/surgery/euthanasia if any these procedures are used in the Protocol.

12. Methodology

Compare the checked boxes with the Lay Description. Are they congruent? Often Protocols will include a description of a procedure such as live capture, bleeding or special diet, but will not include the correct check marks in this Section.

a. **Field Observation Only:** This refers only to field work where no animals will be handled as part of the Protocol. This type of study may require a wildlife permit, depending on the location.

b. **Live Capture of Wild Animals:** Usually requires an appropriate wildlife permit. Is one included? If this box is checked, the restraint section must be included. However, restraint information should not be included here.

c. **Tissue, Organ, Device, Etc. Collection:** Includes any animal tissue or organ harvested pre- or post-mortem. Includes blood collected post-mortem or under terminal anesthesia. Does not include blood collected pre-mortem. Includes harvest of implanted devices. Includes collection of any animal by-product including fur, feathers, saliva, milk, feces, etc…

d. **Fasting and/or Water Deprivation:** Includes any variation from the feeding/watering schedule typical for the species involved (e.g., *ad libitum* food and water for rodents). This includes reduced food or water, if less than 85% of the typical ration is provided. Includes fasting/water restriction prior to surgery. The rationale for the restriction and the duration and effects of the deprivation must be provided.

e. **Special Diet/Water:** Are details of special diet or water provided?

f. **Restraint of Freedom of Movement While Awake:** This must be completed:
   i. If wild animals are captured (any method, any duration)

ii. If the restraint will be painful (any duration)
iii. For restraint that lasts longer than 15 minutes

If a laboratory animal is picked up and held for less than 15 minutes, this does not qualify as restraint. Farm animals housed in USDA approved housing (e.g., standard calf crates, farrowing crates) do not qualify as restraint.

Ensure that details of the restraint method, duration and monitoring are included. For field trapping, this includes details for the actual trapping and any handling post-trapping. If the animal is to be transported to another location, it includes details on the temporary housing, the duration and monitoring, as well as the disposition of the animal following transportation. A separate restraint section is required per species, unless the restraint is **exactly** the same for all species, which should be stated as such.

g. **Special Housing or Husbandry Needs:** This is completed if the animal requires special caging (e.g., metabolic cages) or additional husbandry within University Animal Care facilities or a satellite facility such as a Campus Ag Center. If the animal is to be housed for greater than 24 hours in the investigator lab, or another non-UAC facility, this section must be checked and the SOP for housing and husbandry must be included.

h. **Bleeding:** This does not include blood collected post-mortem (see above). The frequency refers to the interval between blood draws (e.g., once a week for three weeks). The volume is the amount per draw. If anesthesia or palliative therapy is used for a blood draw, the drugs/method used must be completed. The total number of blood draws is per animal over the duration of the animal's use.

i. **Tail-Tipping:** If boxes 2 or 3 are checked, ensure there is a justification and the anesthesia option is completed.

j. **Toe Clipping:** Please note that toe clipping should only be used only when no other method of identification is feasible. In this case, strong justification, including the age of the animal must be included.

k. **Immunization/Antibody/Ascites Production and Collection:** This section is completed for vaccination, antiserum production or monoclonal antibody protocols.

l. **Inoculation of Biological Materials of Mammalian-Origin into Rodents:** The material to be inoculated must:

i. Be of mammalian origin
ii. Must be inoculated into rodents (mice, rats, hamsters, gerbils, guinea pigs or chinchillas)

It does not include microorganisms (unless grown in tissues/cell culture). In this case, they should be included under "Other" and list the host tissue as well as the microorganism (e.g., Vaccinia virus grown in monkey kidney epithelial cells).

m. **Test Drug Administration:** Any drug used for standard veterinary care (palliative therapy, anesthesia/sedation, surgery) or euthanasia should not be included here (these should be listed in the appropriate sections below). If the study includes the use of non-standard drugs for palliative therapy (such as in a pain study), or if any other drug or compound is administered to the animal in any form (oral, topical, parenteral, inhalation), it should be included here.

Includes any hazardous drugs or compounds listed in the Hazardous Materials Addendum. Does not include microorganisms or physical agents (UV light, irradiation, etc…). The source of any experimental drug should be included.

n. **Palliative Therapy for NON-SURGICAL Procedures with Pain Category of C-E:** This section must be completed only if palliative therapy is given. The box should not be checked if no palliative therapy will be given. This is only

completed if any of the animals are in Category C, D or E. Category E animals may receive some palliative therapy if part of the manipulation is under a different Category.

Example
A rat receives an injection of tumor cells with lidocaine (topical anesthetic) as palliative therapy. The protocol then requires that the tumor develop without any palliative therapy (E).

A mouse undergoes surgery to cause tissue damage with post-surgical analgesia for 48 hours (Category D). The tissue damage is allowed to persist for 3 weeks without any palliative therapy (Category E).

In both cases the animals are placed in Category E as the most painful Category.

o. **Anesthesia and/or Sedation for Non-Surgical, Survival Procedures:** This section is completed if the animal will be sedated or anesthetized for non-surgical procedures such as imaging (MRI) or irradiation (Cobalt 60), injections, biopsies, etc… If the animal will be terminated while under anesthesia (e.g., perfusion and removal of heart for tissue harvest) this is not a survival procedure and the drug details/method should be included under euthanasia.

p. **No Palliative Therapy Offered for NON-SURGICAL Procedures with Pain Category of C-E:** If no palliative therapy is offered to Category C, D or E animals, this section must be completed. This includes animals anesthetized for non-invasive procedures such as bleeding and/or imaging (both Category D for the anesthesia).

Justification must be given for withholding palliative therapy for Pain Categories of C-E. In the case of anesthesia for non-invasive procedures, the justification is that the procedures are minor and cause not more than momentary pain. For other procedures, there must be strong justification for withholding palliative therapy. In the case where animals undergo multiple procedures, justification for withholding palliative therapy for all procedures must also be given (e.g., surgical implantation of tumor cells [procedure 1], followed by development of the tumor [procedure 2]).

q. **Euthanasia:** All the methods that will be used must be listed. It is acceptable to list multiple methods for one species, if necessary. If multiple species are listed on the protocol, provide the method for each species. If all the species will undergo the same euthanasia, then this should be clearly stated.

13. **Use of Pharmaceutical Drugs, Gasses and Chemical Compounds**

Investigators should use pharmaceutical grade drugs, gasses and chemical compounds whenever possible. Exceptions to the rule are possible:

a. If the use occurs on animals being terminated (i.e., non- pharmaceutical grade CO_2 for euthanasia)
b. If there is scientific necessity
c. If an acceptable veterinary or human pharmaceutical-grade compound is not available

There must be specific review and approval for the use of all non-pharmaceutical compounds by the IACUC.

14. **Clinical Signs and Criteria for Moribundity**

Clinical Signs: All the signs that could occur from the Protocol procedures, even if unlikely should be listed, including clinical signs that may arise a function of the strain of animal used (e.g., spontaneous tumor formation, early death, etc…). Regardless of whether clinical signs are likely or unlikely, a reason for the clinical signs should be provided. "Not applicable" as a clinical sign is acceptable for field studies where animals will not be handled and or for animals that will only be euthanized for tissue harvest (i.e., generally only Category A animals).
Criteria for Moribundity: These criteria are specifically designed to assist investigators, University Animal Care staff and Veterinarians in deciding whether an animal merits immediate euthanasia for humane reasons. These signs may

arise as a function of the study or may occur spontaneously. Even if clinical signs are unlikely from the Protocol procedures, Criteria for Moribundity must be provided. The only protocol where no Criteria for Moribundity is acceptable is one with only field observation (i.e., animals are never handled).

15. **Release of Confidential Information**

Not relevant to the review process.

16. **Principal Investigator Assurance and Signatures**

The principle investigator and the department head/director must sign and date this section. The IACUC Office may have a signed copy of this part of the Protocol, however, absence of signatures should be noted.