Institutional Animal Care and Use Committee

New Protocol Review Form - Research

Last updated 12/1/2013

Section 1: General Information

Protocol Information

IACUC Protocol Number: _____
Protocol Title: _____
Previous IACUC Protocol Number: _____
(For studies that are continuations.)

Office Use:
Approval Date: _____
Expiration Date: _____

Principal Investigators

	Name
Lead Principal Investigator (PI): *[Note: Only a faculty member may be listed as lead PI.]*	_____

Primary Contact Information

Contact Person: _____
Department: _____
Interoffice Mail Code: _____
Phone Number: _____

Alternate Contact Information (to be used in emergencies when the primary contact is unavailable)

Alternate Contact Person: _____
Department: _____
Interoffice Mail Code: _____
Phone Number: _____

Directions:

- **Important:** Save this form to your computer's hard drive before completing it, or your responses may not be captured!
- It is recommended that you read the entire form before completing.
- This form must be completed and submitted (as a Word document) electronically. Submit to the online system. Retain a copy of your completed form for your records.
- Please respond to all questions in this form. If a particular question does not apply to your study, please indicate this. Type responses in the designated shaded boxes or check the designated check boxes.
- Download and complete all relevant appendices.
- For questions, contact the IACUC Administrative Office at IACUC@vt.edu or 540/231-0931.

Section 2: Assessment of Unnecessary Duplication

References:
Section 2:

The Guide: Page 25-26
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 research and its review by the IACUC:

- unnecessary duplication of experiments

AWA: § 2.32 - Personnel qualifications.
(c) Training and instruction of personnel must include guidance in at least the following areas:
(5) Utilization of services (e.g., National Agricultural Library, National Library of Medicine) available to provide information:
(i) On appropriate methods of animal care and use;
(ii) On alternatives to the use of live animals in research;
(iii) That could prevent unintended and unnecessary duplication of research involving animals; and
(iv) Regarding the intent and requirements of the Act.

1. If this study is an extension of previous work, briefly explain why more work needs to be done.

- **If not an extension = N/A**
- **If this is a continuation protocol why is it needed:**
  - **Unable to complete the original protocol in three years**
  - **Completed the original protocol but the data requires additional experiments for new questions.**

EXAMPLE: The current protocol is an extension of protocol XYZ. In protocol XYZ we completed 3 of the 4 experiments proposed. In this protocol we propose to complete experiment 4 and followup on the results of experiments 1 – 3.

2. Provide a narrative description of how you came to the conclusion that this study does not unnecessarily duplicate previous work (e.g., current/ongoing scientific literature assessments, recent scientific meetings, consultation with peers).

- **The narrative should clearly state this is not unnecessarily duplicating previous work.**
- **Provide the mechanism in which the above has been determined (examples):**
  - **Review of literature – provide search terms**
  - **Recent relevant scientific meetings**
  - **Discussion with colleagues**

EXAMPLE: A PubMed search using the search terms " ____," "____," and "____" followed by a review of the results and attendance at recent scientific meetings clearly demonstrate that the experiments proposed in this protocol do not unnecessarily duplicate previous research.

Section 3: Justification of Species Selection

References for Sections 3 and 4:

The Guide: page 25-26
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 animal
- 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; see Appendix A, Experimental Design and Statistics)

Animal Welfare Act Regulations 2.31
(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;

1. List the type of animals to be used on this protocol.
    - **All strains or species described in Section 9 below or the online "Animal" section should be listed.**

Species or Strain

2. Justify the selection of species (check all applicable boxes).
    - **All applicable boxes should be checked and the boxes checked should match the explanation found under question #3.**

☐ This is a new model.
☐ A large database exists for this species, which will allow comparisons with previous data.
☐ The anatomy, genetics, physiology, or behavior of the species is uniquely suited to the study.
☐ This is the phylogenetically lowest species with adequate size, tissue, or anatomy for the study.
☐ The results will be directly applicable to the health or care of this species.
☐ Other (please describe): _____

3. Explain why a "lower order species" or non-animal alternatives cannot be used to achieve the desired results.

    - **Brief explanation of why the species proposed was chosen.**
    - **Rationale as to why a lower order species has been chosen**
    - **State why an in vitro or other non-animal alternative cannot be used.**

EXAMPLE:

1. **Species-specific research – This study must use pigs because we are examining the effect of diets with varying protein/carbohydrate/fiber ratios on meat/fat ratio in market swine and results from a lower species would not be applicable.**
2. **Study moving from cell culture to live animals – Mice were chosen for the current study because we have validated the ability of compound "X" to kill tumor cells in vitro using mammary tumor cell lines and now we need to validate if effectiveness in a live animal model. Nude mice will serve as the host for mammary tumors for testing compound "X."**

Section 4: Justification of Number of Animals

A key principle in the ethical use of animals in research, testing, and teaching is that the number of animals used in each project is the minimum necessary to obtain valid and meaningful results. In determining the numbers of animals required, which of the following are applicable:

- **One or more boxes can be checked below but be sure that answer matches the Experimental design in Section 9 and if there are multiple experiments or species described that they all scenarios are represented in the justification.**
  - **Examples:**
    - **If there are five experiments using mice and all five use the same n value then only description is required. But if two experiments use a different n value both n values should be described below (could be two power analyses or one power analysis and one pilot).**
    - **If more then one species on the protocol all species should be justified in the number of animals the PI has requested.**

☐ A statistical assessment (power analysis) was performed. Describe the statistical method/test used to determine appropriate group sizes/animal numbers, and state if a statistician was consulted to assist in the determination:

- **Power Analysis:**
  - **What is the power the PI wants to achieve?**
  - **What N value is required to achieve this power?**
  - **Outcome variables taken into consideration?**
  - **Description on how the power analysis was performed?**

☐ The numbers of animals or group sizes have been established by federal guidelines/requirements.

- **State and describe which federal guideline/requirement they are following under "other."**

☐ This is a pilot study that uses the minimum number of animals required to provide meaningful, but not statistically significant, data.

- **Review the number of animals requested to ensure it represents a pilot study.**
  - **Example if the PI is requesting greater then 10 animals per group this may not be a pilot study as the number should represent a minimum number that will not provide statistically significant data.**

☐ Other (please describe):

- **Example:**
  - **Study may not have been done in this species before or in any species and the PI references other similar studies in determining the N value.**
    - **In this case often times you can still request the power that was achieved in the referenced articles.**

Section 5: Pain Category

References

VA Tech Guideline: Guidance Concerning USDA Pain and Distress Category Assessments in IACUC Protocols

Animal Welfare Act: Annual Report: 2.36 (b) (5-8)
(5) State the common names and the numbers of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs. Routine procedures (e.g., injections, tattooing, blood sampling) should be reported with this group;
(6) State the common names and the numbers of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used;
(7) State the common names and the numbers of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used shall be attached to the annual report;
(8) State the common names and the numbers of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.

Please indicate which pain category your study will be in, based on the descriptions below. If animals will undergo procedures in more than one pain category, list only the highest category.

Pain Category: **After review of the protocol, in particular Sections 9 and 10, review each of the pain categories below and match the highest pain level described within the protocol with pain category description below. If you are not an agreement with the PI on the pain level then request the PI to upgrade and provide the appropriate justification if required (Section 7 alternative search for D and E and Section 6 for E's). (Multiple pain levels can be on one protocol.)**

Pain Category Descriptions

B: Breeding or holding animals only; no research conducted.

C: Use of procedures that cause no or slight/momentary pain or distress (e.g., observational studies; injection of non-irritating agents; blood collection from peripheral vessels; collection of cells or tissues following euthanasia).

D: Use of procedures that would cause more than slight/momentary pain or distress, but are performed using appropriate anesthetics, analgesics, or tranquillizers to relieve pain (e.g., minor or major surgical procedures [survival or non-survival] performed under anesthesia; collection of cells or tissues prior to euthanasia; painful procedures performed under anesthesia [retro-orbital blood collection in rodents]).

E: Use of procedures that cause more than slight/momentary pain or distress, but that cannot be performed using anesthetics, analgesics, or tranquilizers without adversely affecting the study (e.g., toxicity and lethal disease studies in which the animals are allowed to die without intervention and mortality is the endpoint). Mechanical restraint may, depending upon duration and type of restraint, be considered a category "E" procedure. **Approval to conduct a Category E study requires detailed justification.**

Section 6: Justification for Category E Procedures

References:

The Guide: page 25-27: Protocol Review

Special Considerations for IACUC Review
Certain animal use protocols include procedures or approaches that require special consideration during the IACUC review process due to their potential for unrelieved pain or distress or other animal welfare concerns. The topics below are some of the most common requiring special IACUC consideration. For these and other areas the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns. By considering opportunities for refinement, the use of appropriate non-animal alternatives, and the use of fewer animals, both the institution and the principal investigator (PI) can begin to address their shared obligations for humane animal care and use.

Animal Welfare Act: Annual Report: 2.36 (b) (7)

(7) State the common names and the numbers of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used shall be attached to the annual report;

If applicable, provide an explanation for the requirement to perform painful or distressful procedures without appropriate pain relieving or sedating medications. Please include scientific references.

- **Determine which procedure in the protocol causes the protocol to be categorized as an E?**
  - **If one procedure or multiple elevates to an E categorization has the PI adequately justified why they cannot use pain relief or sedation for one or all?**
  - **Has the PI justified the requirement to perform the procedure?**
  - **Literature citations of why pain relief cannot be utilized.**

EXAMPLE: Vaccine testing – In the present study pain-relieving drugs cannot be given because animals must be allowed to exhibit symptoms of disease to validate the effectiveness of our experimental vaccine. Any treatment of disease would make it impossible for us to evaluate our vaccine.

Section 7: Evaluation of Alternatives

References:

Animal Welfare Act:

- **2143 Standards and certification process for humane handling, care, treatment, and transportation of animals**
  - **2143**
    - **(a)(3)(B): that the principal investigatory considers alternatives to any procedure likely to produce pain to or distress in an experimental animal;**
    - **(a)(7)(B)(i): information on procedures likely to produce pain or distress in any animal and assurances demonstrating that the principal investigatory considered alternatives to those procedures;**
- **Institutional Animal Care and Use Committee: 2.31(d)(1)(ii): The principal investigatory 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;**
- **Annual Report 2.36(b)(2): Assure that each principal investigator considered alternatives to painful procedures;**

The Guide:

Key Concepts (page 3)

- Throughout the *Guide,* scientists and institutions are encouraged to give careful and deliberate thought to the decision to use animals, taking consideration the contribution that such use will make to new knowledge, ethical concerns, and the availability of alternatives to animals use (NRC 1992).

Regulations, Policies, and Principles (page 12)

- Consideration of alternatives (in vitro systems, computer simulations, and/or mathematical models) to reduce or replace the use of animals

Protocol Review (page 25)

- Availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A, Alternatives)

Additional citations throughout the *Guide*

The Federal Animal Welfare Act and PHS Policy require that researchers evaluate the existence of alternatives when procedures cause more than slight or momentary pain or distress for the animal. Examples of alternatives include less sentient animal models, computer models, audio-visual training programs, and refinements to proposed procedures.

The Virginia Tech IACUC requires that an alternatives search be conducted for studies in pain categories D and E. If applicable, complete the following questions:

1. Which database sources or methods were used to evaluate the existence of viable alternatives? You must **include two or more databases** in your search.

   ☐ AGRICOLA ☐ Biological Abstracts

☐ Current Contents Connect
☐ VetCD (Index Veterinarius)
☐ PubMed (Medline)
☐ Other(s) (please identify): _____

- **A minimum of two databases should be checked.**

2. Give the key words used in conducting the database search and number of hits. ie anesthesia and mouse and lupus number of hits 512, etc.

- **Use of the word "alternative" should be used. Depending on the study other search terms the might be used include "model," "vitro," "culture," "simulation"**
- **Search for the procedures or disease that cause the pain or distress to be a category D or E.**

Example #1: Protocol using retro-orbital blood collection

Blood collection – 35,871
Blood collection AND mouse – 994
Blood collection AND mouse AND techniques – 7
Blood collection AND mouse AND alternatives – 7
Blood collection AND mouse AND anesthesia - 37
Blood collection AND retro-orbital – 21
Blood collection AND retro-orbital AND alternatives – 0

Example: Protocol studying *Haemophilus* vaccine efficacy in swine
Vaccine – 248997
Vaccine AND efficacy – 22893
Vaccine AND efficacy AND swine – 263775
Vaccine AND *Haemophilus* - 5036
Vaccine AND efficacy AND *Haemophilus* – 476
Vaccine AND efficacy AND *Haemophilus* AND swine – 19
vaccine AND vitro AND haemophilous AND pig - 9
vaccine AND haemophilus AND pig AND alternative – 2
vaccine AND haemophilus AND pig AND model – 18
vaccine AND haemophilus AND pig AND pain OR distress - 0

3. Date(s) when the search was conducted:
   - **Inspectors give the guidance that this search should not have occurred on the same day the protocol was submitted because it does not show the PI had ample time to perform a thorough search and review the literature.**
   - **The search should have been completed within three months of submission of the protocol.**

4. Years included in the search criteria:
   - **Dates should be current and go back minimally to the previous protocol's search or if a new search as far back as the database can search.**

5. What alternatives (replacements, reductions, and refinements) were identified?

   NOTE: the use of pain relieving drugs for surgical and other procedures is considered a refinement.

   - **Replacement, reduction and refinement should be considered and addressed?**

- **Are they applying the Three R's in their protocol but have not listed them as identified alternatives? If yes and they have not remind them to list in this section.**

Example #1 for retro-orbital eye bleed – use of anesthesia would be a refinement. Alternatives would be submandibular or jugular vein blood collection. If not chosen should be justified below.

- **If the PI has not listed alternatives that you are aware of request they list and explain why not being used in question #6.**
- **Should be a narrative statement that discusses alternatives.**

Example #2 for vaccine efficacy: "Based on my years of experience in this field and periodic consultation of bibliographic sources outlined above, I believe there is no alternative to performing vaccine efficacy trials were identified to achieve the scientific objectives of this research. Therefore, based on the aforementioned references, this procedure is the most appropriate for conducting my research."

6. If alternatives were identified but will not be used, please provide justification.
   - **Did they provide adequate justification of why they cannot use the alternative? Literature citations?**
   - **If a justification is available under Section 6 (Justification of Category E procedures) reference see section 6 above for justification.**

Example: The search for alternatives to retro-orbital blood collection in mice identified jugular vein collection and submandibular collection. These techniques will not be used because…….(need justification for not using other techniques).

The following are "red flags" used by regulatory agencies to determine that searches were insufficient:
1. Only one (1) database was searched.
2. Search does not include terms for the painful procedure itself (*e.g.,* craniotomy, uveitis).
3. Search terms included only for painful, but not otherwise distressful, aspects.
4. The term "alternative" was used alone with no other alternative terms (*e.g.*, analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare).
5. Keywords were not relevant to the protocol.
6. Keywords and concepts were linked incorrectly.
7. An inadequate time period was searched (< 5 years).

Section 8: Animal Well-Being & Harm/Benefit Analysis

References:

#1 The Guide Page 24-26:

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:

- impact of the proposed procedures on the animals' well-being

#2 Harm-benefit analysis

On page 27, the *Guide for the Care and Use of Laboratory Animals* (NRC 2011) indicates that for studies that have the potential for unrelieved pain or distress, there are special considerations for IACUC review. Specifically, the *Guide* indicates that "the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns." This seems to indicate that for studies involving the potential for pain and distress, the IACUC should conduct a "harm/benefit" analysis. What does AAALAC expect with regard to Committee evaluation of these kinds of studies?

The 2011 *Guide* specifies that the Committee is obliged to weigh study objectives against animal welfare concerns in accordance with the tenets of the Three R's. This analysis is typically already performed by IACUCs in their reviews of proposed animal studies. AAALAC International expects that IACUC's (or comparable oversight body), as part of the protocol review process, will weigh the potential adverse effects of the study against the potential benefits that are likely to accrue as a result of the research. This analysis should be performed prior to the final approval of the protocol, and should be a primary consideration in the review process. For studies potentially involving unrelieved pain and distress, the AAALAC International site visitors will assess whether the Committee has conducted this analysis.

1. Explain how you will minimize expected animal pain and distress and enhance animal well-being (e.g., use of sedatives, tranquilizers, or anesthetics; familiarization/conditioning of the animal; enrichment opportunities).

- **In the response have they adequately described how the pain and distress of the animal will be minimized?**
- **Have enrichment opportunities for the animal's well-being been described?**

2. Please assess whether the "harm" caused to the research animals is justified, please explain the benefits to humans or animals or both from the proposed research activity that would outweigh the "harm".

- **Has the PI adequately justified the harm of the animal for the benefit of the research activity?**
- **Has the PI described how this research will provide benefit down the road for the species being utilized and/or the species the research may translate to in the future.**

Section 9: Experimental Design

References:

#1 Animal Welfare Act:

2.31(d): IACUC review of activities involving animals.

#2 The Guide Page 24-26:

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:

- a clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee

Describe the experimental design of this study. Please include the following for each experiment if you have more then one on this protocol:
1. Experiment number (ie 1, 2, 3) and brief description (two sentences),
2. Treatment groups and number of animals in each group,
3. Total number of animals to be used given as an equation to include replicates and extra/replacement animals. ex. 4 treatment groups x 10 animals per group x 2 doses x 2 replicates = 160 animals + 2 extra animals as back up (only if needed),
4. A time line of the experiment to start on day of acquisition of the animal to end of experiment giving only the name of the procedure to be done and the description will be in Section 10. (You may attach your timeline to the submission and state "see attached time line".) ex on approximate day -14 animals arrive, day 0 Dependent on treatment group treatment given Day 0-28 monitored Day 1, 5, 8, 10, 14, 20 and 28 blood drawn, fecal sample, physical, Day 28 euthanized.

- **Has the PI responded to all four questions above and:**
  - **Have they given a brief description for each experiment and does the description correlate to the information given in responses to questions 2-4?**
  - **Are the treatment group numbers inline with the N value given under animal number justification in Section 4?**
    - **Or is this a pilot and if yes is the N being used reasonable for a pilot?**
  - **Can I understand the animals experience from the time it enters the protocol until the end?**
    - **Are the procedures on the timeline described under Section10 #1 and conversely are the procedures in Section 10 #1 shown on the timeline?**
    - **Are the appropriate Appendixes completed for what is described on the timeline (example Appendix A filled for samples) and conversely are the items listed on the Appendixes described on the timeline or within the treatment groups and for both does the information match?**
      - **For example if there are 6 blood draws on the timelines 2 days apart is the same described in Appendix A and vice versa?**
  - **Does the last day of the timeline define the disposition of the animal? Ie euthanized, transferred, etc?**
- **The most important item for this section is can you understand the animals' experience.**

Section 10: Experimental Procedures

1. Please list and describe all procedures identified in Section 9 and give any possible adverse effects they may have on animal health or well-being. *For surgical procedures please only give possible

adverse effects and say "see appendix B for description." *Attach any monitoring sheets that will be used on this protocol at submission to IACUC.

- **Has the PI described all of the procedures listed on the timeline(s) in Section 9 and given any possible adverse effects.**
- **How descriptions can be written:**
  - **Describe procedure in enough detail to understand the animals experience and that anyone could understand the description enough to perform the procedure.**
  - **Reference an SOP attached under Supporting Documents (if OUV SOP's any deviation from the SOP will be written in this section.)**
  - **Reference Appendix B "Surgical" for surgical procedures.**
- **Euthanasia should not be described here, it will be described in Section 14.**

2. Sedation/Anesthesia/Euthanasia/Analgesia Agents: Please complete the following table in order of when the agents will be used during the protocol. Under purpose please state if the agent will be used for pre-anesthetic, sedation, etc.

- **This section should include any sedation/anesthesia/euthanasia/analgesia referenced in Section 9, 10 question #1 and Section 14 #4.**

Agent	Dose (mg/kg)	Route of Administration	Frequency of Administration	Purpose

References:

#1 Animal Welfare Act:

2.31(d)(x): (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;

#2 The Guide Page 30:

Multiple Survival Surgical Procedures

Surgical procedures in the laboratory setting may be categorized as major or minor (USDA 1985). Whether a procedure is major or minor should be evaluated on a case-by-case basis, as determined by the veterinarian and IACUC (NRC 2003b; Silverman et al. 2007; for additional discussion see Chapter 4, Surgical Procedures). Regardless of classification, multiple surgical procedures on a single animal should be evaluated to determine their impact on the animal's wellbeing. Multiple major surgical procedures on a single animal are acceptable only if they are (1) included in and essential components of a single research project or protocol, (2) scientifically justified by the investigator, or (3) necessary for clinical reasons. Conservation of scarce animal resources may justify the conduct of multiple major surgeries on a single animal, but the application of such a practice on a single animal used in separate protocols is discouraged and should be reviewed critically by the IACUC. When applicable, the IO must submit a request to the USDA/APHIS and receive approval in order to allow a regulated animal to undergo multiple major survival surgical procedures in separate unrelated research protocols (USDA 1985, 1997a). Justifications for allowing animals not regulated by the USDA to undergo multiple survival procedures that meet the above criteria should conform to those required for regulated species. If multiple survival surgery is approved, the IACUC should pay particular attention to animal well-being through continuing evaluation of outcomes. Cost savings alone is not an adequate reason for performing multiple major survival surgical procedures.

Some procedures characterized as minor may induce substantial postprocedural pain or impairment and should similarly be scientifically justified if performed more than once in a single animal.

3. Please refer to the information below to determine if your study involves multiple major survival surgeries, and mark the appropriate response.

A major surgery is defined as one that penetrates and exposes a body cavity (e.g., abdomen, thorax, or skull) or produces substantial impairment of physical or physiologic functions. Multiple survival surgeries involve completion of a surgery from which the animal recovers from anesthesia, and then a subsequent surgery from which the animal recovers from anesthesia.

☐ This study involves multiple major survival surgeries. *Respond to question 4, and then go to question 5.*
☐ This study does not involve multiple major survival surgeries. *Go to question 5.*

4. Provide a scientific justification for the need of multiple major surgeries. Please note that the IACUC can approve multiple major survival surgeries only if there is an adequate scientific justification for the surgeries, the surgeries are required as part of routine veterinary care, or the attending veterinarian has determined that the surgeries are necessary to protect the health or well-being of the animal.

- **Determine if multiple major surgeries will be occurring?**
  - **Has the PI justified the requirement to perform multiple surgeries?**
  - **Has the PI included literature citations?**
  - **Has the PI performed an alternative search of the procedure under section 7?**
  - **If this is a USDA animal a written request with support data has to be sent to APHIS for final approval before the protocol can be approved.**

References

VA Tech Guideline: Guidance Concerning USDA Pain and Distress Category Assessments in IACUC Protocols

Animal Welfare Act: Annual Report: 2.36 (b) (5-8)
(5) State the common names and the numbers of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs. Routine procedures (e.g., injections, tattooing, blood sampling) should be reported with this group;
(6) State the common names and the numbers of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used;
(7) State the common names and the numbers of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used shall be attached to the annual report;
(8) State the common names and the numbers of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.

5. Indicate if death will be used as an endpoint in this study. Use of death as an endpoint refers to situations in which an animal is allowed to progress to death as a required experimental outcome. It does not refer to situations in which an animal is euthanized at an established humane endpoint.

☐ Death will be used as an endpoint in this study. *Respond to question 6, and then go to Section 11.*
☐ Death will not be used as an endpoint in this study. *Go to Section 11.*

6. Use of death as an experimental endpoint is strongly discouraged. If possible, a moribund (ie lateral recumbency, little or no response to external stimuli) condition or other non-death endpoints should be used as alternatives. If this study requires the use of death as an experimental endpoint, provide a scientific justification for this need. The IACUC cannot approve the use of death as an endpoint without adequate scientific justification.

- **Determine which procedure will cause death as an endpoint?**
  - **If one procedure or multiple procedures will cause death as an endpoint must determine if the PI adequately justified the need for death as an endpoint?**
  - **Has the PI included literature citations?**
  - **Has the PI performed an alternative search of the procedure under section 7?**
  - **Protocol will be categorized as an E and specific clear monitoring intervals must be included in the protocol.**

Section 11: Humane Endpoints

References

Animal Welfare Act: Institutional Animal Care and Use Committee (IACUC): 2.31 (d) (1)(i and v)
(i) Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals;
(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;

The Guide:
Page 12: U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training

- establishment of humane endpoints

Page 25: Protocol Review

- description and rationale for anticipated or selected endpoints
- criteria and process for timely intervention, removal of animal from a study, or euthanasia if painful or stressful outcomes are anticipated

Page 27: Experimental and Humane Endpoints

- The experimental endpoint of a stud occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress, including death. The humane endpoint should be relevant and reliable (Hendriksen and Steen 2000; Olfert and Godson 2000; Sass 2000; Stokes 2002). For many invasive experiments, the experimental and humane endpoints are closely linked (Wallace 2000) and should be carefully considered during IACUC protocol review. While all studies should employ endpoints that are humane, studies that commonly require special consideration include those that involve tumor models, infectious diseases, vaccine challenge, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, organ or system failure, and models of cardiovascular shock.
- The PI, who has precise knowledge of both the objectives of the study and the proposed model, should identify, explain, and include in the animal use protocol a study endpoint that is both humane and scientifically sound. The identification of humane endpoints is often challenging, however, because multiple factors must be weighed, including the model, species (and sometimes strain or stock), animal health status, study objectives, institutional policy, regulatory requirements, and occasionally conflicting scientific literature. Determination of humane endpoints should involve the PI, the veterinarian, and the IACUC, and should be defined when possible before the start of the study (Olfert and Godson 2000; Stokes 2000). Information that is critical to the IACUC's assessment of appropriate endpoint consideration in a protocol includes precise definition of the humane endpoint (including assessment criteria), the frequency of animal observation, training of personnel responsible for assessment and recognition of the humane endpoint, and the response required upon reaching the humane endpoint. An understanding of preemptive euthanasia (Toth 2000), behavioral or physiologic definitions of the moribund state (ibid.), and the use of studyspecific animal assessment records (Morton 2000; Paster et al. 2009) can aid the PI and IACUC when considering or developing proposed endpoints. When novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC, and veterinarian. A system for communication with the IACUC should be in place both during and after such studies. Numerous publications address specific proposals for the application and use of humane endpoints (e.g.,CCAC 1998; ILAR 2000; OECD 1999; Toth 1997; UKCCCR 1997).

1. Even if pain or distress are not anticipated in your study, you must establish humane endpoints at which animals will be removed from the study, treated, or euthanized should unexpected complications arise. Examples of appropriate criteria might include a weight loss limit as a percentage of initial or expected body weight, allowable durations of anorexia, allowable tumor size or total tumor burden expressed as a percentage of body weight, the presence of health problems refractory to medical intervention, and severe psychological disturbances. Describe the humane endpoint criteria that will be used for determining when sick animals, **both on and off study**, will be euthanized or otherwise removed from the study. *Attach any monitoring sheets that will be used on this protocol at submission to IACUC.

    - **Have the humane endpoints been clearly identified?**
        - **Do the endpoints include:**

        - **A scoring system: Does the scoring system identify what action to be taken once a score is reached? For example: initiate increased monitoring, contact a veterinarian, euthanasia.**
        - **If no scoring system are humane endpoints specific and clear. For example, if the animal reaches a 15% weight loss they will be euthanized.**
    - **If there are multiple experiments on the protocol are the humane endpoints the same for each experiment?**
        - **If not, are the endpoints clearly defined and separated by experiment?**
    - **If there are parameters to measured or specific frequency of monitoring ensure that the information given here corresponds to information given: in section 9 timelines, section 10 description of procedures and Appendix B and D. These areas can also reference part of their descriptions by stating See Section 11 for specific details on humane endpoints and monitoring. But any measurements must be described under Section 10 #2 and monitoring frequency if only described under section 11 must be clear in frequency and initiation of additional monitoring.**
- **Veterinary consultation: If decisions will be based from veterinary consultation clearly describe when a veterinarian will be called.**
- **If there are monitoring parameters a monitoring sheet should be uploaded under supporting documents. Compare the monitoring form to the humane endpoints parameters described in this section to ensure they match.**

2. The IACUC requires the PI or a designated staff member to monitor animals that have developed significant signs of illness or toxicity **every four hours, 24 hours per day, including weekends and holidays**. Please provide contact information for the personnel who will be responsible for monitoring the condition of the animals.

    - **Anyone listed below should also be included in the online section 2 Personnel.**
    - **The individual should be appropriately trained and have access to the animals to be able to assess the animals.**

Name	Virginia Tech PID *(Typically the part of an official VT e-mail address that precedes @vt.edu.)*	Phone Number

Section 12: Study Personnel

References

The Guide: Has multiple references to appropriate training of personnel throughout. Page 15 specifically discusses Personnel Management: Training and Education: First paragraph:

- All personnel involved with the care and use of animals must be adequately educated, trained, and/or qualified in basic principles of laboratory animal science to help ensure high-quality science and animal well-being. The number and qualifications of personnel required to conduct and support a Program depend on several factors, including the type and size of the institution, the administrative structure for providing adequate animal care, the characteristics of the physical plant, the number and species of animals maintained, and the nature of the research, testing, teaching, and production activities. Institutions are responsible for providing appropriate resources to support personnel training (Anderson 2007), and the IACUC is responsible for providing oversight and for evaluating the effectiveness of the training program (Foshay and Tinkey 2007). All Program personnel training should be documented.

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- adequacy of training and experience of personnel in the procedures used, and roles and responsibilities of the personnel involved

Animal Welfare Act:
Institutional Animal Care and Use Committee (IACUC): 2.31 (d) (1)(viii)
(viii) Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures;

1. If any personnel do not have experience with the exact species and procedures indicated, please describe how they will be trained. Include information about the qualifications of the person providing the training.

    - **By checking the box the training regime represents the minimal amount of training that is required by the IACUC for personnel without experience to complete.**
    - **Other: This box should be evaluated to determine if it meets the minimum required training above and the additional value the training that will be provided will bring to the competency of the trainee.**

    ☐ Minimally the trainees will observe the procedures three times or until comfortable to perform, then they will minimally perform the procedure three times supervised or until the trainer feels that they are competent to perform the procedure unsupervised. At that point they can perform the procedure unsupervised.

    ☐ Other: _____

2. Who will order the animals for this protocol? (Only check a or b if applicable.)
    a. ☐ CVM
    b. ☐ LAR (includes purchases for VTCRI, LS1, ILSB, etc)

    - **One of the above boxes should be checked to correlate with the facility that will be used for housing the species requested.**
    - **If neither box is checked, and animals are to be acquired for the protocol review the housing and husbandry document Section 2 #1 source to ensure an appropriate source**

has been identified. Additionally, if it is an outside source the Office of the University Veterinarian may have additional questions to establish health status of the animals and if the source of animals is compliant with federal regulations when applicable.

Section 13: Animal Housing and Husbandry

References

The Guide:

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- nonstandard housing and husbandry requirements
- a clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee

Throughout the guide there are standards. Within the sections it describes exceptions and how they may be granted a few examples:

- Pg. 64: Single housing of social species should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being.
- Pg. 116: Unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery should be conducted in dedicated facilities or spaces.
- Appendix B: U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
    - IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

Animal Welfare Act:

Annual Report: 2.36 (3)

(3) Assure that the facility is adhering to the standards and regulations under the Act, and that it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the IACUC. A summary of all such exceptions must be attached to the facility's annual report. In addition to identifying the IACUC-approved exceptions, this summary must include a brief explanation of the exceptions, as well as the species and number of animals affected;

Miscellaneous:2.38 (k) (1)

(1) Each research facility shall comply in all respects with the regulations set forth in subpart C of this part and the standards set forth in part 3 of this subchapter for the humane handling, care, treatment, housing, and transportation of animals; *Provided, however,* That exceptions to the standards in part 3 and the provisions of subpart C of this part may be made only when such exceptions are specified and justified in the proposal to conduct the activity and are approved by the IACUC.

1. Indicate if the animals involved in this study have been or will be used in other experiments or instructional labs. (Check all that apply.)

☐ The animals involved in this study have not been and will not be used in other experiments or instruction. *Go to question 3.*
☐ The animals involved in this study have been used in prior experiments or instruction. *Respond to question 2, and then go to question 3.*
☐ The animals involved in this study will be used in concurrent experiments or instruction. *Respond to question 2, and then go to question 3.*
☐ The animals involved in this study may be transferred to another experiment or instructional protocol. *Respond to question 2, and then go to question 3.*

- **Reasons to check each box above and reminder more than one box may apply:**
    - **Box 1: The animals will only be used on this protocol and either euthanized or adopted at the conclusion.**
    - **Box 2: The animals have been used on other IACUC approved protocols before coming to this protocol. (This does not include if an animal was weaned from a breeding protocol). Answer question 2 stating a general description of the protocol the animal had previously been on and if possible the IACUC protocol number.**
    - **Box 3: The animals will be used on more than one IACUC approved protocol simultaneously. Answer question 2 stating the protocol numbers that the animal will be simultaneously participating on while on this protocol and general**

information of the procedures that will be performed on those concurrent protocols.

- **Box 4: At the conclusion of this protocol the animal may be transferred to another IACUC approved protocol. Answer question 2 by stating which animals can be transferred to another IACUC approved protocol maybe only naïve or ones that have not completed the experiment and will be transferred to the continuation protocol of the current protocol or ?**

2. Please describe prior, concurrent or future use of these animals, and what measures will be taken to prevent over-use.

- **Please see above information has been provided for each box for information that should be provided dependent on the box checked.**
- **Additionally, if boxes 2-4 are checked the PI should clearly define how overuse will be prevented. If this is through rest periods the periods should be included in the timeline provided in Section 9 or if they are Per the CVM rest periods endorsed by the IACUC this should be stated.**

3. Are special or unusual housing or husbandry conditions required for the animals involved in this study?

☐ Special or unusual housing or husbandry conditions are required. *Respond to questions 4 and 5, and then go section 14.*
☐ There are no special or unusual housing or husbandry requirements. *Go to section 14.*

- **Box 1: This box should be checked if there is mention of any of the items described under #4 or "other" items that are out of the normal for housing and husbandry of the animals on the protocol and described in the protocol, appendixes or housing/husbandry document.**
- **Box 2: This box will not be checked if none of the items under #4 apply to this protocol or "other" items that are out of the normal for housing and husbandry of the animals on the protocol.**

4. Please indicate what type of special or unusual housing or husbandry conditions are required:

☐ food restriction
☐ water restriction
☐ single housing of social animals
☐ longer than brief restraint
☐ environmental temperatures outside of established ranges
☐ captive housing of wild-caught species
☐ other; *describe in question 5*

- **Check any of the above boxes for special or unusual conditions required within the protocol and describe in more detail under #5.**
- **The "other" box is for any condition that is beyond the standard care for housing and husbandry that is not described in the boxes above. A detailed description of this request will be under question #5.**

5. Please describe the necessity for special or unusual housing or husbandry requirements, and how maximum possible animal comfort will be assured under the conditions.

- **If any of the boxes are checked above the following should be answered for each "condition":**
  - **The condition and description**
  - **Why the condition is required (may need scientific justification).**
  - **Minimum and maximum length of time for the condition.**
  - **When necessary if the condition will cause possible stress to the animal how this will be alleviated. For example: Single housing of social housing stress will be alleviated by allowing the animal to have visual and auditory contact with animals of the same species.**

Section 14: Animal Disposal

References

AVMA Guidelines for the Euthanasia of Animals: Current Edition entire guidelines apply.

The Guide:
Euthanasia: Page 123:

Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the *AVMA Guidelines on Euthanasia* (AVMA 2007 or later editions). In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; irreversibility; time required to induce unconsciousness; appropriateness for the species and age of the animal; compatibility with research objectives; and the safety of and emotional effect on personnel.

Euthanasia may be planned and necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments. Criteria for euthanasia include protocol-specific endpoints (such as degree of a physical or behavioral deficit or tumor size) that will enable a prompt decision by the veterinarian and the investigator to ensure that the endpoint is humane and, whenever possible, the scientific objective of the protocol is achieved (see Chapter 2). Standardized methods of euthanasia that are predictable and controllable should be developed and approved by the AV and IACUC. Euthanasia should be carried out in a manner that avoids animal distress. Automated systems for controlled and staged delivery of inhalants may offer advantages for species killed frequently or in large numbers, such as rodents (McIntyre et al. 2007). Special consideration should be given to euthanasia of fetuses and larval life forms depending on species and gestational age (Artwohl et al. 2006).

The selection of specific agents and methods for euthanasia will depend on the species involved, the animal's age, and the objectives of the protocol. Generally, chemical agents (e.g., barbiturates, nonexplosive inhalant anesthetics) are preferable to physical methods (e.g., cervical dislocation, decapitation, use of a penetrating captive bolt); however, scientific considerations may preclude the use of chemical agents for some protocols. Although carbon dioxide (CO_2) is a commonly used method for rodent euthanasia, there is ongoing controversy about its aversive characteristics as an inhalant euthanasia agent. This is an area of active research (Conlee et al. 2005; Danneman et al. 1997; Hackbarth et al. 2000; Kirkden et al. 2008; Leach et al. 2002; Niel et al. 2008) and further study is needed to optimize the methods for CO_2 euthanasia in rodents (Hawkins et al. 2006). The acceptability of CO_2 as a euthanasia agent for small rodents should be evaluated as new data become available. Furthermore, because neonatal rodents are resistant to the hypoxia-inducing effects of CO_2 and require longer exposure times to the agent (Artwohl et al. 2006), alternative methods should be considered (e.g., injection with chemical agents, cervical dislocation, or decapitation; Klaunberg et al. 2004; Pritchett-Corning 2009). It is essential that euthanasia be performed by personnel skilled in methods for the species in question and in a professional and compassionate manner. Special attention is required to ensure proficiency when a physical method of euthanasia is used. Death must be confirmed by personnel trained to recognize cessation of vital signs in the species being euthanized. A secondary method of euthanasia (e.g., thoracotomy or exsanguination) can be also used to ensure death. All methods of euthanasia should be reviewed and approved by the veterinarian and IACUC.

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- method of euthanasia or disposition of animals, including planning for care of long-lived species after study completion

Animal Welfare Act: Institutional Animal Care and Use Committee (IACUC): 2.31 (d) (1)(xi) and 2.31 (d)(5)
(xi) Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, § 1.1 of this subchapter, unless a deviation is justified for scientific reasons, in writing, by the investigator.
(5) A description of any euthanasia method to be used.

1. Will animals be euthanized during or at the conclusion of this study? (check all that apply)

☐ Animals will not be euthanized. *Respond to question 2, then section completed.*
☐ Animals will be euthanized. *Go to question 3.*

- **Check box 1 if animals will not be euthanized while on the protocol.**
- **Check box 2 if animals will be euthanized while on the protocol.**
- **In some instances both boxes may need to be checked.**
- **When answering the question consider the disposition of the animals at the end of each experiment described on the protocol.**

2. Describe how animals will be disposed of at the conclusion of this study (e.g., transferred to another approved study, maintained in a research or teaching herd).

- **If only box 2 above was checked then this question does not require an answer.**
- **If box 1 was checked then the answer should be reviewed to ensure "reuse" of the animal after the experiment(s)/procedure they underwent is appropriate.**

3. Will animals undergo experimental treatments or procedures prior to euthanasia?

☐ Animals will not undergo treatments or procedures prior to euthanasia (e.g., tissue harvesting).
☐ Animals will be euthanized after experimental treatments or procedures.

- **Box 1 should be checked if the animals will not undergo any procedures or experiments before euthanasia. The most used example of this would be for tissue harvesting. The animal is euthanized immediately and tissues harvested.**
- **Box 2 should be checked if the animals are given any substances, used for any procedures or fed special diets before euthanasia.**
- **In some situations both boxes may need to be checked.**
- **When answering the question consider the disposition of the animals at the end of each experiment described on the protocol.**

4. Describe the method of euthanasia that will be used. Please note that chemical methods of euthanasia are preferable to physical methods. If you plan to use a physical method of euthanasia, you will need to provide adequate justification for this choice (include published references). For injectable agents, include information on dose and route of administration in Section 10 question #2.

- **Methods in this section should be consistent with the current AVMA Guidelines on Euthanasia.**
- **Any physical methods used without anesthesia, methods outside of the AVMA guidelines or methods with conditions from the AVMA guidelines will need to be justified through scientific justification including published references.**
    - **The justification should correlate with the scientific needs of the protocol and scientifically show that use of the AVMA approved methods would impact the data collected.**
- **If any form of decapitation is used as primary or secondary (under question #5 below) the protocol must state: how it is determined the blade is sharp, how the blade is cleaned before the procedure and between animals on the same day, how the sharpness**

of the blade is determined if more than one decapitation is performed during the day and how the blade is sharpened and clearly state if scissors or a guillotine will be used.

5. Describe how the personnel performing the euthanasia will confirm that death has occurred. Examples include: thoracotomy under anesthesia, exsanguination under anesthesia, prolonged exposure to carbon dioxide (greater than five minutes) with cessation of vital signs, and observation of cessation of vital signs for five minutes.

    - **If the original method was decapitation, anesthesia followed by perfusion or anesthesia followed by thoracotomy or a similar method that clearly confirms death then this question can state see response under #4 above.**
    - **If confirmation is required the preferred method is to follow a chemical method with a physical method. However, this is a not always possible dependent on the research or species. Then method utilized should be consistent with death determination including the time to ensure death has occurred.**

6. How will animal carcasses be disposed of (e.g., rendering, landfill)?

    - **Recommend stating per VT EHS disposal as the disposal of animals can periodically changes. However, the PI would then be responsible for ensuring the method of disposal is per VT EHS disposal.**
    - **If the disposal is per landfill ensure that the animal has not been treated with any infectious, hazardous or biological substance as this can/will impact wildlife and should not be disposed of through the landfill.**
    - **Any questions concerning this area contact EHS for confirmation of appropriate disposal.**