Animal Care and Use Committee
INSTRUCTIONS
Protocol Application for the Use of Animals

This instruction manual is to be used when completing an ACUC protocol application.

Please remember that ALL protocol and amendment submissions are due on the 15th of each month for review at the next month's ACUC meeting (held on the first Wednesday of every month). Upon receipt of protocols, an administrative and veterinary pre-review are conducted.

MAIN FORM

GENERAL INSTRUCTIONS

- All sections are expandable, so please insert as much text as needed (the only restriction is that the lay summary should be ≤250 words).
- If a question is not applicable to your study, type N/A.
- Please be conscientious and write all sections for non-specialist readers, define any essential technical terms and acronyms, and correct all grammatical errors and typos.
- Use of references: *new!*
    - The citation MUST include all pertinent information such that the references can be easily looked up using a database such as PubMed.
    - If a citation is used to reference a procedure, the procedure specifics still must be included in the appropriate section or procedural appendices.

PROCEDURAL APPENDICES

- Read the list of available appendices (more detailed information/instructions for these appendices are in these instructions beginning on page 9).
- Check "YES" for those appendices that are applicable to your protocol and that will be completed and attached to the end of the IACUC Protocol Application for the Use of Animals.
- Check "NO" for those appendices that are NOT applicable to your protocol.
- "YES" or "NO" MUST be checked for EACH appendix.
- Complete only those appendices related to your work.
- **Do not return blank or non-required appendices**.
- Return the entire application to the Office of Research Integrity and Compliance via email to:

ACUC@mail.wvu.edu or mhollander@mail.wvu.edu

1. PRINCIPAL INVESTIGATOR ASSURANCE

- Read, sign and date this assurance page to indicate that you will follow all applicable Federal and Institutional Policies and Guidelines.
- This page must be signed by the Principal Investigator OR the protocol can be submitted from the

PI's WVU email address. If the PI chooses to sign the document, a hard copy of only the one signature page needs to be sent to IACUC Office, PO Box 6845. Initials or signature stamps will **not** be accepted.

- West Virginia University has a legal obligation and a written Assurance, on file with the Public Health Service (PHS), which commits WVU to following the standards established by the Animal Welfare Act and NIH Policy. In line with the Assurance, WVU has established the Institutional Animal Care and Use Committee to review all proposals involving animals to ascertain if proposals are consistent with NIH Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Education, the *Guide for the Care and Use of Laboratory Animals*, the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, the Animal Welfare Act, and other applicable public laws and regulations. These documents describe requirements that must be met for humane care and use of research and teaching animals, to assure that animals do not suffer unnecessary distress, pain, disease or injury, and that animals receive proper care and husbandry. All research and teaching animals must be cared for and used in a manner that complies with the above requirements to protect the institution and safeguard animal privileges. Laws further protect worker health and safety and safe handling of potentially hazardous or injurious materials.
- A complete updated ACUC protocol must be submitted ***every three years*** and progress reports must be submitted upon application for renewal of ACUC protocols.

2. GENERAL INFORMATION

- Include all contact information for the PI
- Include all contact information for an alternate contact person if different from the PI
- List project title (if this is an academic course, please include the course number in the title)
- Type of protocol
    - Indicate whether this protocol is for teaching, research, service or maintenance (or some combination of these).
        - Teaching protocol**s**
            - If teaching, the project title should be the name of the course and course number, if course number is known.
            - If this is a TEACHING protocol, note that each time the course is offered, you are required to submit to the IACUC a document that (a) lists the enrolled students; (b) certifies that they will receive animal use orientation training as part of the course OR that they will be instructed to complete the online CITI training; and (c) certifies that the students have been instructed to complete the online Animal Health Questionnaire. This document can be submitted up to 3 weeks after the beginning of the course and can be submitted to the IACUC office via email (ACUC@mail.wvu.edu). An email reminder will be sent to instructors at the beginning of each semester.
            - If instructors do not have students complete the online CITI training and instruct students on regulatory issues and species-specific issues regarding animal care and use, the instructor **must** submit documentation that includes the topics/information covered in this in-class training.
        - Research protocols

        - Research protocols are protocols that involve any type of research categories such as biomedical, behavioral, agricultural, wildlife, etc.
    - Service protocols
        - Service protocols are for those projects offering services using animals (e.g., imaging, inhalation, microscopy, transgenic, etc.) to other laboratories.
    - Maintenance protocols
        - Maintenance protocols are for maintaining breeding colonies, holding animals while no studies are being done on them, veterinary care maintenance, etc.
- Indicate protocol classification
    - New – A protocol for a new animal project that you have not had a protocol for in the past.
    - Renewal – A protocol that is a continuation of a current project for which the current animal protocol has expired or will expire soon (all protocols expire after 3 years). A progress report is REQUIRED for renewals. Failure to provide a progress report may delay review of your protocol.
    - Amendment – An addition, deletion or change to your current protocol. Depending on the nature of the amendment, the review process may require full committee review (FCR).
- Indicate federal, state, foundation, or internal funding source by name (e.g., NIH, NICHD, AHA, RDG, VA, DoD, etc.)
    - Be sure to include the grant number and/or OSP (Office of Sponsored Programs) number, active funding period dates and an indication as to the status of the grant (pending or active).

3. PERSONNEL

- List all individuals who will be working with animals and their department. Individuals must have completed all required training before beginning work with animals. Information regarding what training is required can be found at http://oric.research.wvu.edu/animal/animal_welfare_training.
    - If you provide training in your laboratory for personnel on your protocol (e.g., training on a particular procedure in your protocol) it is important that you document this and keep these records.
- You must also list the procedures which will be done by each of the individuals listed. The PI will be responsible for ensuring that all laboratory personnel are adequately trained in the listed procedures, although it is not required that the PI be the one who trains their personnel. Please attach rows to the table if more personnel are to be listed.

4. PERSONNEL QUALIFICATIONS

A. For all those personnel (including the PI) listed on this protocol who will be responsible for conducting the procedures or for training others on how to perform the procedures to be used in this protocol, a brief summary of their qualifications must be provided. Be sure to include:
    - Name and degree
    - Procedures that they will be responsible for and/or be teaching to others on the protocol
    - What their qualifications are

- Provide information regarding relevant experience; and, if applicable
- Relevant and published manuscripts using the procedure/technique

B. All personnel listed on a protocol must be enrolled in an Occupational Health and Safety Program.
   - If all personnel listed on your protocol are enrolled in the WVU Occupational Health and Safety Program, which is accomplished by completing the Animal Health Questionnaire, check "Yes".
   - If you answer "No", you must indicate who is not enrolled and why. If they are covered under an Occupational Health and Safety Program at another institution, please be sure to indicate that.
   - If you know of any people who will be in close proximity of the work you will be doing with/on animals but are not listed on this protocol, PLEASE contact the IACUC office via email or at 304-293-3968. It is very important that WVU and the IACUC identify ALL personnel who may need to work around animals so that a risk assessment can be completed to be sure that allergies, etc. will not impact their health.

C. You may also include a request for additional, more specific training that the IACUC, OLAR or Farm Staff can arrange.

5. ANIMAL INFORMATION

A. "YES" or "NO" MUST be check for each of the procedures listed here.
   - Check "YES" for those procedures that will be relevant to your study.
     - If "YES" is chosen for any of the listed procedures, it is very likely that at least some of your animals will be listed under category D or E. The definitions of each of the pain categories can be found in these instructions on page 5.
   - Check "NO" for those procedures that will not be part of your study.
   - If you propose to perform a procedure that may lead to pain or distress that is not listed here, please indicate that next to "Other".

B. Species #1: Include all information for the first species to be listed on your protocol.
   - Indicate the scientific and/or common name of the species.
   - Indicate the strain/stock/breed of the species.
   - Indicate the source of this species.
   - Indicate the gender(s) of the species needed.
   - Indicate the age or weight of the species.
   - Indicate the number of animals needed for the entire term of the protocol (the longest a protocol can be active before it must be completely renewed is 3 years).
     - This number should include the animals that are obtained from breeding (beginning from the day of birth), even if you discard them before your study (e.g., if they have the incorrect genotype).
     - This number should be the sum of the animals numbers for all categories.
   - Animals must be assigned to a pain category. Definitions of the pain categories are provided below.
     - You may have animals in one or all of the pain categories depending on what animals or groups of animals are undergoing what procedures.
     - Indicate the number of animals that will be used for each category (when the

numbers from all the categories are added up, they will equal the number that was indicated in the section "Number of Animals for the length of this protocol").
- A justification for the chosen pain category(ies) is required.
- The IACUC has the right to change these categories as they see fit.

C. Species #2: Include all information for the second species to be listed on your protocol.
- Instructions listed above for Species #1 can be followed.
- If you have more than two species, complete Appendix N as many times as needed.

D. Check "Yes" if you have more than two species and will be submitting an Appendix N to add more species. Check "No" if you will not be submitting an Appendix N.

DEFINITIONS of USDA PAIN AND DISTRESS CATEGORIES:

B – Animals housed for breeding or other purposes where no experimental manipulations are required.

C – Animals will **not** undergo procedures or experience conditions that would normally cause more than momentary or slight pain or distress. Note that euthanasia before significant pain and distress could be C. Include all animals that will be euthanized because they fall under the category of "wastage" (e.g., the wrong genotype) in this category.

D – Animals may potentially experience more than momentary or slight pain or distress and will receive some corrective measures, such as anesthetics, analgesics, or tranquilizers during or after the procedure to prevent more than minor pain or distress. **Use this category if euthanasia is delayed until the onset of significant pain or distress**.

E* – Animals may potentially experience more than momentary or slight pain or distress and will not receive a corrective measure, such as anesthetic, analgesics or tranquilizers or other therapies to alleviate pain or distress. One possibility is if euthanasia is purposefully delayed for scientific reasons until significant pain or distress has occurred.

*For category E animals, justification on scientific ground is required, please complete Appendix G.

PLEASE NOTE: The IACUC has the right to change the pain category assignments as they see necessary.

6. HOLDING AND PROCEDURE ROOM INFORMATION

A. Check all the buildings where animals will be held or housed. This refers to housing only, NOT locations where procedures will be conducted.

B. List the buildings where animal procedures will be done along with the room numbers and what procedures will be done in particular locations.

7. LAY SUMMARY

- A summary of the proposed use of animals must be included such that a lay person can understand the basic problem you will be researching.
- Provide a statement indicating the benefits of your research to society, scientific understanding or animal welfare.
- Avoid technical words, jargon, undefined abbreviations and acronyms, and words such as "kill" or "sacrifice".
- It is not necessary to justify the use of specific species in the lay summary, you may want to remove specific species names and use the general term "animal".
- Remove references to personal names and to articles.
- Remove all typos.
- We advise you to write this summary at an 8th grade level and as if it will end up in the public domain.
- Please keep this at ≤250 words.
- Keep in mind that this lay summary may be available to the public through the Freedom of Information Act and that the use of species names is not required.

8. EXPERIMENTAL DESIGN

A. Scientific description

- Give a brief but clear and concise sequential description of the project and procedures involving the use of animals that is easily understood by all members of the IACUC.
- **PLEASE NOTE!! Include an overview of the experiment here and do NOT include procedural details here. Procedural details need to be put into the appropriate appendices.**
- Include:
  - Specific objectives, aims, or goals
  - An outline of the experimental groups or conditions in the project (including animal numbers and endpoints), if possible, use a flow diagram - a brief description of each group or condition should be provided here. **NOTE: A sequential schema to explain the steps each animal will be exposed to throughout the experiment/study is REQUIRED.**
  - Provide details in appropriate appendices (It will help the Committee tremendously if, as you describe each group or condition, you indicate the letter of the appendix that contains the relevant details.

B. Justify the number of animals being requested.

- Detail how the numbers of experimental animals were obtained (convenience or cost cannot be used as a justification) using a table and/or a format such as: Experiment 1 – A x B x C x D = F (total animals). Justify the numbers using one or more of the following:
- Group size determined statistically – provide power analysis, including power, variance or standard deviation and effect size.
- Group size determined based on project being a "pilot" study. The number of animals allowed for pilot studies may be limited by the committee.
- Group size based on quantity of harvested cells or amount of tissue required.
- Group size based on a referenced peer-reviewed paper with a similar study design and

outcomes that establishes group sizes.

- BREEDERS: For breeders where litter sizes and usable young are unknown, make reasonable estimates. Do not count animals as breeders and as experimental animals, but DO count the conservatively predicted number of progeny that will be culled or used experimentally. NOTE that ALL animals will be counted at birth rather than at weaning!
- FIELD STUDIES: For field mark-recapture or population studies, list the largest number you could catch or manipulate. For example, (no traps) x (# trap nights) x (% full traps/night) x (3 years) = total number of animals.
- TEACHING: For teaching, describe the numbers based on class size, procedures/lab periods. Previous or projected class sizes can be used. For example, (*number of class sections) x (*animals per lab class) x (times class taught in 3 years) = total number of animals. *Base these numbers on max size.
- The following articles may be helpful for this section:
  - D. Fitts, "Minimizing Animal Numbers: The Variable-Criteria Sequential Stopping Rule", *Comparative Medicine*, 61(3): 206-218, 2011.
  - D. Fitts, "Ethics and Animal Numbers: Informal Analyses, Uncertain Sample Sizes, Inefficient Replications, and Type I Errors", *Journal of American Association for Laboratory Animal Science*, 50(4): 445-453, 2011.

9. EUTHANASIA

A. The primary method(s) of euthanasia need to be listed here. The termination of experimental animals, when necessary, must be accomplished in a humane manner and by acceptable techniques as recommended by the AVMA Guidelines on Euthanasia, 2007.

- If the method is purely a physical method, describe this method in the space provided.
- If the method is purely chemical, list the relevant information in the table. Dose ranges are recommended to provide flexibility but not required. If you are uncertain about dosage information for a particular drug/agent or species, please consult the veterinary staff.
- If the method is a combination of the two, use the space provided as well as the table.

B. A secondary method of euthanasia to ensure death must be included. Examples are thoracotomy, bilateral pneumothorax, exsanguination, cervical dislocation, removal of a vital organ, etc.

C. Provide scientific justification if the euthanasia method your study requires is **not** described as "acceptable" by the AVMA Guidelines on Euthanasia, 2007.

- Include specific information and justification as to why an acceptable method cannot be used. In any case, you must also provide a statement detailing the experience of the personnel that will be performing this euthanasia.
- If you will be using an AVMA acceptable method, please list N/A in this section.

D. If animals will not be euthanized at the end of a study, include a description of what will be done with the animals.

- If animals will be adopted out, there is an adoption form that you can complete and submit. Please contact OLAR or the IACUC office in ORIC for this form.
- If animals will be transferred to another protocol and the animals are housed in OLAR

facilities, please make sure to fill out an Animal Transfer form from OLAR.
- Animals that are to be returned to the wild require special consideration and approval by the IACUC.

10. IACUC POLICIES, PROCEDURES AND GUIDANCE DOCUMENTS

PLEASE NOTE that there are ACUC policies being added all the time. Be sure to ALWAYS use the most current version of the protocol form. The most current version will always be found on the ORIC website, http://oric.research.wvu.edu/animal.

A. You must check one of the three options, "YES", "NO", or "N/A", for EACH policy, guideline, or standard operating procedure (SOP).
   - Check "YES" for each policy, guideline, and SOP that are applicable to your study. All of these documents can be found at http://oric.research.wvu.edu/animal/guidlines.
   - If you cannot follow an approved policy, guideline or SOP, check "NO".
   - If the policy, guideline, or SOP is not applicable to your study, check "N/A".

B. This section lists IACUC-approved TEMPLATES for standard operating procedures. If your protocol involves any of these, you must create and include an SOP specific for your study/lab using these available templates.
   - Check "YES" for each SOP template that is applicable to your study. All of these documents can be found at http://oric.research.wvu.edu/animal/guidlines.
   - If you cannot use an IACUC-approved template, check "NO".
   - If the SOP template is not applicable to your study, check "N/A".

C. If "NO" was checked for any of the IACUC-approved policies, guidelines or SOPs, you must justify why they cannot be followed or used. Details that would otherwise be covered in these documents must, therefore, be specified in your protocol.

D. If you have checked "YES" for any of the IACUC-approved policies, guidelines or SOPs, it is imperative that you read the document AND follow it.
   - Check "Agree" if you have read the applicable documents and agree that you will follow them as written.
   - Check "Disagree" if you have not read the applicable documents and/or you will not agree to follow them as written. Please note that checking "Disagree" will likely hold up approval of your protocol.

11. REFINEMENT, REDUCTION AND REFINEMENT

A. Replacement – Consideration of non-animal alternatives or animals lower on the phylogenetic scale must be stated.

   1. Describe the reasons and justify why the project should use animals rather than non-animal alternatives such as in vitro systems, human clinical trials, computer models, etc.
   2. Justify the species you will be using based on literature, previous studies, unique anatomy, prior data, genetic or physiologic characteristics, etc.

B. Reduction – Efforts MUST be made to minimize the numbers of animals utilized in animal studies.

1. Indicate if this project will unnecessarily duplicate previous work. If duplication is necessary, please justify (e.g., coursework).

C. Refinement – If your project may cause more than momentary pain or distress (i.e., if your protocol includes animals under pain category D or E), this section is required. Efforts MUST be made to improve procedures and methods to reduce animal numbers or reduce variability in data, morbidity or mortality.

It is a Federal requirement that two databases be searched for alternatives to painful/distressful procedures that are being used. For help see http://www.nal.usda.gov/awic/alternatives/tips.htm.

1. The search to be completed in section 11.C.2 must be a search for alternative to potentially painful or distressful procedures. This first question is to assist you with identifying these procedures for which alternatives should be searched for.
2. Be sure to fill out all sections of the chart indicating that the required searches were completed.
   - You must search at least **two** databases and indicate:
     1. The dates searched
     2. Boolean operators that were used
     3. The number of hits for each.
3. You must indicate if alternatives to the procedures that you listed in section 11.C.1 were found.
4. Regardless of whether you found alternatives or not, you MUST summarize the results from your search and indicate why alternatives that may have been found will not suffice.

APPENDICES

A. Appendix A – Surgery Management of Surgical Pain and Distress

If your project requires surgery of any kind, this appendix must be completed.

1. Indicate the category of surgery
   - Non-survival surgery – animal will be euthanized before it recovers from anesthesia
   - Survival surgery – animal will recover from anesthesia and regain consciousness
   - Multiple survival surgeries – animal will receive more than one survival surgery and this INCLUDES surgeries that had been done on the animal before arrival at WVU

2. If you will be performing surgery on mice or rats, check the box indicating that you will follow the "Rat and Mouse Surgery WVU" IACUC policy. If you will be performing surgery on any other type of animal, check the box indicating that you will follow the "Peri-Operative Care of USDA Regulated Species" IACUC policy.

3. Give a detailed description of the surgical procedure. Information should include (but is not

limited to):

1. All information that is specific to this surgical procedure.
2. Anesthetics, analgesics, or tranquilizers that will be used. Include specifics of these drugs in appendix C.
3. Specific information regarding wound closure and, if applicable, suture type and pattern.

4. Give a detailed description of the care that will be provided during and after the surgical procedure. Information should include (but is not limited to):
    1. How the depth of anesthesia will be determined.
    2. The frequency and duration of monitoring.
    3. Analgesics given. If post-operative analgesics will not be given, this must be justified.
    4. Criteria for determining that the animal has recovered.
    5. Potential problems that will be watched for.

5. Give a detailed description of the methods that will be used to detect and evaluate pain and distress in animals. Information should include (but is not limited to):
    1. Intraoperative monitoring techniques.
    2. Steps that will be taken to avoid or minimize pain or distress to animals.
    3. Criteria used to determine when animals should be euthanized (i.e., endpoint criteria).

6. Give your best estimate regarding possible percentages for morbidity and/or mortality due to the proposed procedures. Information should include (but is not limited to):
    1. A description explaining this percentage.
    2. Likely causes for morbidity and/or mortality and how they will be addressed.

7. If multiple surgeries will be done on an animal, there must be an explanation and justification.

B. Appendix B – Other Non-surgical Procedures

Details of any non-surgical procedures should be included in this appendix.

Procedures involving ***prolonged restraint of unanesthetized animals or food/water deprivation must be justified***. Be sure to detail the procedure and also include duration, frequency, monitoring, adaptation training, purpose, and removal criteria.

1. Identify each non-surgical procedure with a name

2. Provide details of the non-surgical procedure.

3. If this non-surgical procedure involves the use of anesthesia, you must describe the anesthesia monitoring and post-procedural care. Information should include (but is not limited to):
    - How depth of anesthesia will be determined.
    - The frequency (during anesthesia and recovery) and duration of monitoring.
    - Analgesics given, if applicable
    - Criteria for determining that the animal has recovered.
    - Potential problems that will be watched for.

4. If there might be morbidity and/or mortality associated with this procedure, indicate the percent possibility that this might occur. Be sure to include likely causes and what steps will be taken to minimize them.

Fill out a separate appendix B for each non-surgical procedure. If you have a multiple step procedure (where each step might be considered a separate procedure), you can include them all under one general name and then list them all on one page. For example: If you are doing multiple behavioral tests to evaluate memory, you might name the procedure "Memory Tests" and then list all the tests that will be done to evaluate memory along with the descriptions of those tests.

C. Appendix C – Use of Drugs in Animals

1. List ALL anesthetics, analgesics, sedatives/tranquilizers, and experimental drugs/chemicals here that will **not** be used for euthanasia (drug information for euthanasia drugs should be in section 9 of the main form). BE SURE to include:
   - Dose or dosage range in **mg/kg**,
   - Route (e.g., IP, SC, IM, inhalation, oral, etc.)
   - Frequency (how often per day)
   - Number of doses
   - Which procedures they pertain to

Hazardous Chemicals

It is the PIs responsibility to determine if each drug/chemical is considered HAZARDOUS to personnel. Chemicals or drugs that are considered hazardous MUST have an accompanying Standard Operating Procedure (SOP). Please see the Use of Hazardous Chemicals in Vivarium Animals Policy when writing these SOPs. This template for writing SOPs can be found at http://oric.research.wvu.edu/animal/guidlines. You may contact the Safety Office for assistance as well. There are some chemicals that already have approved SOPs and these can be found at the above link as well.

Non-Pharmaceutical Grade Compounds

If you are using non-pharmaceutical grade compounds, be SURE to review the Policy for Non-Pharmaceutical and Pharmaceutical Grade Compounds Used.

D. Appendix D – Collection of Blood, Tissue and Body Fluids

This appendix should be completed if bodily fluids (e.g., such as blood or urine) or tissues are to be collected or if no procedures will be done to the animals except for euthanasia for the purposes of collecting tissue.

1. List the tissue and/or body fluids that will be collected from the animal.
2. If a tissue or fluid is being collected from the animal multiple times (e.g., blood collection from saphenous vein once a week, collecting fecal samples daily, muscle biopsies, etc.), you must indicate that frequency of collection.
3. The volume of blood being collected must be indicated and if the volume is > 1% of body weight, a justification is also required.
4. Method (e.g., aspiration) and site (e.g., tail vein) must be indicated.

5. If the tissue or fluid will be collected while the animal is under anesthesia or sedation, appendix C must be completed to include the information for the anesthetic or sedative.
6. Indicate if the animal will be euthanized before or after tissue collection. If the animal will NOT be euthanized, be sure to mark, not applicable.

E. Appendix E – Breeding Animals

This appendix must be completed if breeding of the animals is being done at WVU and is part of the research project. Please note that many questions in this appendix will pertain mostly to rodent breeding. If you will be breeding other species (e.g., sheep, cattle, etc.), please indicate N/A for those question that do not apply.

Describe your breeding plan by answering all of the questions.

1. Indicate the type of breeding will be done.
   - One male x one female – with regards to rodent breeding, you must make an assurance that either the female will be removed from the male before she gives birth OR that the older litter will be removed before a second litter is delivered (i.e., you must assume that a pregnancy will result from post-partum estrus).
   - Harem breeding (one male x 2-4 females) – with regards to rodents breeding, you must make an assurance that the females will be removed from the cage BEFORE they give birth. If you leave a female with the male, this becomes one on one breeding and you must then follow the guidelines given in the section above.
   - Artificial Insemination
   - Other
2. Describe the management of your breeding strategies to ensure that all applicable policies are followed (e.g., Breeding and Weaning of Rats and Mice at WVU, Rodent Counting Guidance).
   - For rodent users – It is IMPERATIVE that there not be more than one litter in a shoebox size cage. Be sure to provide a plan here for how you will avoid this and be sure to follow that plan.
3. Rodent Users – Estimate, the best you can, how many breeding pairs will be used for each strain of animals to maintain the line of animals. It would be helpful to indicate the number of males versus females, especially if you will be harem breeding.
4. Rodent Users – Indicate how many litters or offspring will be produced based on the number of breedings done. Keep in mind that all breedings may not be successful. You may also answer this question using a percentage based on the number of breedings.
5. Indicate approximately how many or what percentage of litters or offspring will be used for experimental purposes.
6. Rodent Users – Indicate approximately how many or what percentage of litters or offspring will be unusable (i.e., wastage animals because of incorrect genotype, etc.).

F. Appendix F – Genetically Modified Animals

If you are working with or generating genetically modified animals (GMAs) at WVU, you must complete this appendix.

Reminder: The creation of genetically-modified animals requires IBC approval and then

***appendix I must also be completed*.**

1. If genetically modified animals will be generated at WVU using the Transgenic Animal Facility (TAF) as part of this protocol, you must:
   - Get approval from the Institutional Biosafety Committee (IBC)
   - Have the TAF submit an amendment for THEIR IACUC protocol to include the new strain that you will be generating (you will need to provide a scientific justification for the creation of this line to the TAF). This may be done subsequent to approval of your protocol, or in tandem.
2. If you will not be creating or generating NEW genetically modified animals at WVU, please indicate the source of the GMAs that you will be using.
3. If you will be breeding GMAs, please indicate as such and complete appendix E.
4. When creating new GMAs, there is a federal mandate to provide a plan as to how you will monitor intended AND unintended phenotypes. Especially phenotypes that have the potential to induce pain or distress.
   - If you KNOW that the genetic manipulation may lead to pain or distress, answer n/a to this question and complete question #5.
   - If you do NOT know if the genetic manipulation leads to pain or distress, you MUST include a plan describing HOW you will monitor the animals to ensure that they are not in pain or distress.
     - How often will the animals be handled and/or looked at?
     - What physical signs of pain or distress will you watch for?
     - If pain or distress does develop and is not due to other factors, what will your plan be for management of that pain or distress? What is the endpoint criteria?
   - If you KNOW that the genetic manipulation does NOT lead to pain or distress because this is an established line, provide this information as a justification for why these genetically modified animals may not require a stringent plan to monitor phenotype.
   - This question does NOT refer to genotype only phenotype.
5. If you KNOW that there are known hazards to the animal and/or personnel handling the animals, be sure to indicate what those hazards are and how they will be monitored or addressed.

G. Appendix G – Pain Category E Justification

Remember that only those animals that will undergo procedures causing more than momentary pain or distress without relief need to be categorized under pain category E. If you have groups of animals (possibly control groups) that will not undergo these procedures, be sure that you assign those animals to the appropriate lower pain category.

1. Justification of ALL category E procedures must be included with your protocol. This appendix MUST be completed if you are proposing a procedure that is considered a category E (more than momentary pain or distress without relief). This justification should include (but is not limited to):
   - A scientific justification for why pain or distress cannot be relieved.
   - Why other non-painful or –distressful procedures cannot be used.
2. A detailed description and justification is required if the endpoint of your study includes death or significant morbidity. This description MUST include monitoring of the animals (how often,

by whom, what will you be watching for, etc.).
3. It is VERY important to be sure that an earlier endpoint cannot be used and to justify this.

H. Appendix H – Physical Restraint

If any physical restraint is required for your study in animals NOT under general anesthesia, you must complete this appendix. This is only for prolonged restraint and would not include momentary restraint for the purposes of things such as IP injections, tail vein injections, vaccinations, shaving, etc.

The definition of physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation.

1. The frequency of restraint must be stated (e.g., how many times per day, week, month, etc. for each animal). If some groups of animals will be restrained more times than other groups, be sure to make this distinction.
2. Specify the time period of restraint. Prolonged restraint should be avoided if possible. However, if physical restraint is necessary for ≥1 hour, justification is required.
3. Describe the exact procedure for the restraint and the plan for monitoring of the animals while they are being restrained.
4. Describe how the stress of restraint will be kept to a minimum. If stress cannot be minimized, a justification must be included.

I. Appendix I – Use of Biological or Infectious Materials in Animals

This appendix must be completed if any biological materials (this includes all reagents of human and animal origin) or infectious materials will be used in animals.

Please consult with the Institutional Biosafety Committee (IBC) to determine if IBC approval is required.

If IBC approval is required, approval of IACUC protocols will not be granted until receipt of this IBC approval. If IBC approval is not required, documentation stating as such must be provided to the IACUC (this may be in the form of an email from the IBC).

A standard operating procedure (SOP) must be included if the "Use of ABSL-2 in Animals SOP" does not apply to the biological material you will be using.

NOTE: The creation of genetically-modified animals requires IBC approval.

J. Appendix J – Use of Radioactive Materials in Animals

This appendix must be completed if any radioactive materials are to be used in animals.

Approval from the Radiation Safety Committee (RSC) is also required.

Be sure to refer to the "Standard Operating Procedures for Usage of Radioactive Materials in Animals".

K. Appendix K – Satellite Housing Request

If animals must be housed for ≥ 24 hours outside of the standard animal facilities, complete this appendix. **Housing animals in a satellite facility requires ACUC approval and a physical inspection of the space.** This appendix is geared more toward animals being used in the OLAR vivaria.

If housing for 12-24 hours outside of the standard animal facilities IACUC approval is still required, but this is not considered satellite housing. Include this information in section 5.C of the main form. See the OLAR website on SOLE for the specific guidelines.

1. Give the exact location of the proposed satellite facility and provide information regarding security of the room and who has access.
2. Environmental stability and monitoring information must be provided. If you are unsure about how or what to monitor, contact OLAR for assistance.
3. Allowance for satellite facilities requires a scientific justification. Neither cost nor convenience can be used as a justification.
4. It is imperative that there be emergency contact personnel provided and posted in case of an emergency. Also, be sure to include a care plan that contains the following information:
    - Who will be caring for the animals
    - Bedding, water and cage change schedule
    - How often the animals will be checked. Note that all animals MUST be observed at least once EVERY day (this includes weekends and holidays). A log MUST be kept in the room to record checks, cage changes, water and food changes, temperature, etc. If you need a sample log, please contact OLAR.

L. Appendix L – Protocol Renewal Progress Report

A progress report is required for any protocol submission that is a renewal of a current protocol. Teaching protocols DO NOT require a progress report.

This progress report must include:

- A narrative describing what work was completed on this project in the past 3 years.
- The number of animals that have been used for this project so far.
- Optional – a list of publications and grants that have come from the work on the current protocol.

M. Appendix M – OLAR Information (for use of HSC, LSB, BRNI vivaria)

This appendix must be completed only if you will be housing animals in any of the OLAR vivaria.

1. Any animals that leave the vivarium and return for housing MUST be housed in a room specifically for animals that go back and forth between the vivarium and the laboratory. MAKE SURE that you indicate if you will be bringing animals to your lab and back to the vivarium and

then contact OLAR to coordinate what room to house these animals in.

2. If animals need to be transported outside of HSC, LSB, or BRNI, please justify. Movement of laboratory research animals from these areas MUST be done in cooperation with OLAR and MUST be approved by OLAR and the ACUC beforehand.
3. If any of your animals require special care while being housed in the vivarium, indicate that here. You will also need to complete a Special Care Request form with OLAR.
    - Note that meal feeding animals to meet their nutritional requirements is NOT considered food restriction or deprivation (for example, feeding sheep a specified amount of food that they consume before a 24 hour period is complete).