Institutional Animal Care and Use Committee (IACUC)
INSTRUCTIONS for
Protocol Application for the Use of Animals

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

Please remember that ALL protocol and amendment submissions take time to process. Category B, C and D protocols are eligible for DMR but may be called for FCR at any point in the review process (defined below). Category E protocols and protocols using paralytics are almost always required to undergo FCR. Submissions should be received on the 15th of the month prior to the IACUC meeting (usually the first Wednesday of every month). Upon receipt, an administrative pre-review is conducted before the protocol is sent on to the designated IACUC member(s) for review. Veterinary review occurs concurrently with IACUC review.

Definitions:

FCR (Full Committee Review) – If FCR is required, approval of those protocols may be granted only after review at a convened meeting of the IACUC with a quorum of members present. Following FCR, members may vote to approve or if modifications are required to secure approval, may send the review of subsequent revisions to DMR (as defined below).

DMR (Designated Member Review) - If FCR is not required, at least one member of the IACUC, designated by the IACUC Chair and qualified to conduct the review, shall review the protocol and have the authority to approve, require modifications (to secure approval), or request FCR of the protocol. These types of protocols are processed as rolling reviews with no set deadlines. However, submission timing should be planned with consideration of the possibility that any IACUC member can call for FCR at any point in the review process and with consideration of the expiration date of the current protocol in the case of renewal applications.

MAIN PROTOCOL FORM

GENERAL

- All sections contain textboxes and are expandable, so insert as much text as needed (the only restriction is that the lay summary must be ≤250 words).
- If a question is not applicable to your protocol, type N/A. (All boxes must have a response)
- Please be conscientious and write all sections for non-specialist readers. Also, define any essential technical terms and acronyms at first use, and correct all grammatical errors and typos.
- When submitting amendments, ensure that all necessary sections and appendices are updated to conform to the amended portion. (see amendment instructions)
- If there are any questions regarding these instructions, please contact the Office of Animal Welfare (OAW) at iacuc@mail.wvu.edu.

PROCEDURAL APPENDICES

- Read the list of available appendices (more detailed information/instructions for these appendices are in these instructions beginning on page 13).
- Check "YES" for those appendices that are applicable to your protocol and that will be completed and attached in your submission email. Check "NO" for those appendices that are NOT applicable to your protocol.
- Submit the entire application by sending an email to the OAW at iacuc@mail.wvu.edu. Include the main form and relevant appendix forms in Word format as individual files (or as a zip file). Do not combine files into one document or convert to a pdf.

1. PRINCIPAL INVESTIGATOR (PI) 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 Principal Investigator's (PI) WVU email address. 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 IACUC to review all proposals involving animals to ascertain if proposals are consistent with the Animal Welfare Act, PHS Policy on Humane Care and Use of Laboratory Animals, National Research Council, the Guide for the Care and Use of Laboratory Animals (the Guide), Federation of Animal Science Societies (FASS) the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Ag Guide), 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, teaching, maintenance, and service 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 IACUC protocol must be submitted every three years (de novo review), and progress reports must be submitted with the application for renewal of IACUC research protocols.

2. GENERAL INFORMATION

- Include all contact information for the PI. The PI must be a WVU faculty member unless otherwise authorized by the IACUC. Special consideration may be given to others with appropriate authority to serve as a PI on a case-by-case basis (e.g. Core Facility Directors, Clinical Veterinarians, etc.).
- Include all contact information for an alternate contact person if different from the PI.
- List project title.
- Indicate whether this protocol is for teaching, research, service or maintenance (or some

combination of these).

- Teaching protocols
  - Please indicate the subject and course number, if known. The project title is recommended to contain the name of the course and course number.
  - Students taking the course must complete the Occupational Health Questionnaire (OHQ) and be cleared through Occupational Medicine in additional to any required online CITI training. The OAW will obtain course rosters and contact students with instructions on how to complete the OHQ and CITI training. Instructors will be provided with updates regarding the student's completion status. Students should not be allowed to participate in aspects of the course involving live animals until all requirements are completed. The PI must certify that he/she will have provided basic species-specific training prior to active animal handling.
  - If instructors do not have students complete the online CITI training and alternatively 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 category 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 animals, etc.) to other laboratories.

- Maintenance protocols
  - Maintenance protocols are for maintaining colonies, herds, and/or flocks, or for temporarily holding animals that are not currently covered under an approved IACUC protocol. No studies are being performed on any animals and only clinically necessary and standard prophylactic veterinary care may be provided.

- Indicate protocol classification
  - New – A protocol for a new animal project that you have not had a protocol for in the past or for any lapsed/expired protocol.
  - Renewal – A protocol that is a continuation of a current project for which the current animal protocol will expire soon. All renewals must be submitted prior to the current protocol expiration date. A progress report (Appendix L) is REQUIRED for research protocol renewals. Failure to provide a progress report may delay review of your protocol.
  - Amendment – An amendment is an addition, deletion or change to your current protocol. See Amendment Instructions.

- Indicate collaborations and funding source
    - Check the box for collaboration if the animal work in this protocol involves collaboration with an external organization such as another university, research institute, etc. If checked, you make be contacted by the OAW for more information.
    - Indicate the federal, state, foundation, or internal funding source by name (e.g., NIH, AHA, DOD, etc.)
    - Be sure to include the grant number and OSP (Office of Sponsored Programs) number (if applicable), and active funding period dates and an indication as to the status of the funding (pending or active).
    - If there is a change in status or funding source, an amendment should be submitted to reflect the changes.

3. PERSONNEL

- List all individuals, including the PI, who will be working with animals and their department. Individuals must have completed all required training (OHQ and CITI) before beginning work with animals. Information regarding required training can be found at https://animal.research.wvu.edu/training.
- List procedures (or procedure type-e.g. injections, surgery, etc.) which will be done by each of the individuals listed including euthanasia. The PI will be responsible for ensuring that all laboratory personnel are adequately trained for the species utilized and listed procedures, although it is not required that the PI be the one who trains their personnel. Add rows to the table if more personnel are to be listed.
    - Training provided in your laboratory for personnel on your protocol (e.g., training on a specific procedure) must be documented and available for review during IACUC inspections and to veterinary staff. It is very important that you maintain these training records and also document any training received by veterinary or animal care staff or through another organization. Individual Training sheets are available on the website listed above.
- You may add or remove personnel from a protocol at any time with an amendment. Be sure all personnel are added to the protocol and properly trained BEFORE they begin any animal work.

4. PERSONNEL QUALIFICATIONS

A. For all those personnel (including 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, degree, and role (e.g. undergraduate or graduate student, post-doc, technician/farm staff, lab/farm manager, teaching assistant, etc.).
    - Qualifications for working with the specific species and specific procedures they will be performing. Provide information regarding relevant animal experience. Please do not list publications.
    - Indicate if there are specific procedures that they will be responsible for teaching to

others on the protocol.

B. All personnel listed on a protocol must be enrolled in the Occupational Health and Safety Program.
    - If you answer "No", you must indicate who is not enrolled and why.
    - If you know of anyone who will be in close proximity to the area(s) where you will be completing the animal work, but are not listed on this protocol, please contact Occupational Medicine. It is very important that WVU and the IACUC identify ALL personnel who may be working near animal use areas, so that a risk assessment can be completed to ensure the presence of animals will not adversely impact their health.

C. You may also request additional, more specific training that the IACUC, OLAR, or Davis College REOC staff can arrange.

5. ANIMAL INFORMATION

A. "YES" or "NO" MUST be checked for each of the procedures listed here. Some may require you to fill out and submit a corresponding appendix.
    - Check "YES" for those procedures that are relevant to your protocol.
    - Check "NO" for those procedures that are NOT relevant to your protocol.

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 (all strains may be listed in the same species chart).
    - Indicate the sex(es) utilized. Use of only one sex should be justified in the experimental design.
    - Indicate the number of animals needed for the entire duration of the protocol (3 years) in each applicable category (see definitions below).
        - You may have animals in one or all of the pain categories depending on what procedures are performed.
        - Animals that are obtained from breeding need to be included, even if they will not be used for the study (e.g., incorrect genotype).
        - The total number should be the sum of the animal numbers for all pain categories.
        - A brief justification for the chosen pain category/categories is required.
        - The IACUC may change these categories as necessary for compliance purposes.

C. Species #2: Include all information for the second species to be listed on your protocol, if applicable.
    - Instructions listed above for Species #1 can be followed.
    - You may choose to use this chart to indicate separate phenotypes that cause pain and distress.
    - If you have more than two species, you can insert another species section (add 5.D Species #3, 5.E Species #4, etc.) or you can complete an Appendix N.

DEFINITIONS of USDA PAIN AND DISTRESS CATEGORIES:

B – Animals used for breeding or other purposes where no experimental manipulations are required. Breeders that undergo procedures such as genotyping should be category C.

C – Animals will undergo procedures or experience conditions that would not normally cause more than momentary or slight pain or distress. Include in this section all animals that will be euthanized because they are considered "extraneous", have the wrong genotype, etc. Some examples of category C procedures may include simple injections, animal tattooing or tagging, behavior tests, noninvasive imaging, etc. Note that euthanasia before significant pain and distress could be category C.

D – Animals may potentially experience more than momentary or slight pain or distress but will receive some corrective measures, such as anesthetics, analgesics, or tranquilizers during or after the procedure to prevent more than minor pain or distress. Both survival and non-survival surgical procedures should be placed in this category. Other examples include disease models where animals may require extra care to minimize pain or distress during disease progression, such as heat support, soft bedding, etc.

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

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

PLEASE REMEMBER: The IACUC may change the pain category assignment as needed for compliance. If you have any questions or concerns regarding pain category assignment, contact the OAW office.

6. HOLDING AND PROCEDURE ROOM INFORMATION

A. Check all the buildings/locations where animals will be held or housed for 24 hours or more (for USDA-regulated species is > 12hrs). This refers to housing only, NOT locations where procedures will be conducted.
    - If satellite housing is selected, fill out Appendix K. Also, note that the IACUC must approve the proposed satellite location PRIOR to animals being placed there, and the site will receive regular visits from veterinary staff.

B. List the buildings/sites where animal procedures will be performed. List each room number, as well as each specific animal procedure or procedure type including euthanasia that will occur there. Locations where surgical procedures may be performed must be indicated separately, and new surgical sites not already approved and not currently on the regular IACUC inspection rotation (every 6 months) must be IACUC inspected prior to use.
    - If you are unsure of specific rooms/sites, please contact OLAR, Davis College Research, Education, Outreach Center (REOC) manager or a member of your

department for more information.
- Do not list rooms where live animals will not be present (e.g. post-mortem tissue processing).

7. LAY SUMMARY

A. Summary of the proposed use of animals
- A summary of the proposed use of animals must be written in such a way so that a lay person can understand the basic objective of your research or teaching. Write this summary at an 8th grade level and as if it will end up in the public domain.
- The summary should also indicate in laymen's terms how animals are used to meet your objectives.
- Avoid technical words, jargon, undefined abbreviations and acronyms, and words such as "kill" or "sacrifice".
- Remove references to personal names and to articles.
- Please keep this at ≤250 words.

B. Benefit statement:
- Provide a statement indicating the benefits of your research to society, scientific understanding or animal welfare.

8. EXPERIMENTAL DESIGN

A. Scientific description
- Give a concise but clear sequential description of the project and procedures involving the use of animals that should be easily understood by all IACUC members. Assume your audience is not an expert in your field. This section should not read like a scientific grant proposal and should focus on the proposed animal use.
- Include:
  - Specific objectives, aims, or goals. Should be to the point and not overly technical.
  - An outline of the experimental groups or conditions in the project that includes study endpoints should be provided. Humane endpoints should be included if applicable.
  - A flow chart or a sequential description of the procedures a given animal/group undergoes from procurement to final disposition for each project/condition should be included here.
  - Provide details of procedures in appropriate appendices (it will help the IACUC if you refer to the appendix that contains the relevant details for each group or condition.) This section is for an overview of the experiment only – procedural details should be placed in the appropriate appendices and not repeated here.

2. Justify the number of animals being requested.
- Describe how the numbers of experimental animals were obtained (convenience,

cost or space cannot be used as a justification), such as: Experiment 1 – A (treatments) x B (time points) x C (concentrations) x D (group size) = E (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.
  - Group size may be based on the PI's previous experience with the proposed model. You may include a reference or peer reviewed paper.
- BREEDERS: For breeders where litter size and usable young are unknown, make reasonable estimates. Do not double 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.
- FIELD STUDIES: For field work, recapture or population studies, list the largest number you could catch or manipulate. For example, (# traps) x (# trap nights) x (% full traps/night) x (3 years) = total number of animals.
  - You should also explain the non-target animals which include any non-study animals directly or indirectly affected by the research. Examples could include the potential to live-capture or euthanize non-target individuals or disturb or disrupt the activities of other species during the research activity. The non-target species lists might include general descriptors such as "all native mammals/fish" rather than an extensive list of individual species.
  - Number of animals cannot exceed the numbers on acquired permits.
- TEACHING: For teaching protocols, describe the numbers based on potential class size, procedures/lab periods, and how many times a procedure can be safely repeated on an individual animal (e.g. 3 students can perform X a maximum of # times on one animal before moving to the next animal). Previous or projected class sizes can be used. For example, (*# 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/DISPOSITION OF ANIMALS

A. The methods of euthanasia need to be indicated in the chart here. The termination of animals, when necessary, must be accomplished in a humane manner and by acceptable techniques as recommended by the AVMA Guidelines on Euthanasia, 2020. Please also see

the IACUC Euthanasia Guidelines at https://animal.research.wvu.edu/policies-and-guidelines. The methods are separated into three different classifications – **Inhalant, Injectable, and Physical.** Check all methods of euthanasia that apply to the protocol, identifying either a secondary method or verification of death where indicated. Each method utilized should only be indicated once in the chart, based on the primary cause of death for that method. For example, if the method is cervical dislocation under isoflurane anesthesia, isoflurane does not also need to be checked in the Inhalant section because cervical dislocation, not isoflurane overdose, is the primary cause of death. Please type only in the textboxes provided.

DEFINITIONS:
Verification of Euthanasia: Death must be confirmed following euthanasia. Verification can include a secondary method (see definition below) and/or a sensory confirmation such as auscultation (verifying lack of heartbeat/respiration), cessation of opercular movements (aquatic species), etc.
Secondary Method: An adjunctive euthanasia method used to ensure death following the primary method. For example, decapitation performed after CO_2 overdose. Certain euthanasia methods *require* a secondary method be performed.

Inhalant:
- **CO_2**: Indicate if using the standard 30-70% chamber vol/min or if Other, indicate what is being used instead (e.g. a different chamber vol/min, drop jar, etc.) and justify. A secondary method must be indicated.
- **Isoflurane**: Indicate if using the standard 4-5% to effect or if Other, indicate what is being used instead and justify. A secondary method must be indicated.
- **Other**: If using an inhalant other than CO_2 or isoflurane, indicate what inhalant is used and provide dose (chamber vol/min or % concentration) and justify. Immersion agents (e.g. MS-222) can be indicated here. A secondary method or other method used for verification of euthanasia must be included.

Injectable: The secondary method or other method used for verification of euthanasia must be included for injectables.
- **Pentobarbital**: Indicate if using the standard dose range per species or if Other, provide the non-standard dose utilized.
- **Other**: If using an injectable other than pentobarbital, indicate what injectable is used and provide dose and route. MS-222 when used as an injectable (i.e. not as an immersion agent) can be indicated here.

Physical Methods:
- **Cervical Dislocation**: When performed under deep anesthesia, provide the anesthesia used, route, and dose. Scientific justification must be provided if no anesthesia is used. A secondary method or verification of euthanasia must be included.
- **Decapitation**: When performed under deep anesthesia, provide the anesthesia used, route, and dose. Scientific justification must be provided if no anesthesia is used.
- **All of the following methods below must be performed under deep anesthesia (or animal being otherwise unconscious, e.g. from electrical stunning)**. Provide the anesthesia used, route, and dose.

- **Exsanguination**: Indicate the route/site of blood removal (e.g. cardiac puncture). and the total volume of blood removed. A secondary method or other method for verification of euthanasia must be included. Exsanguination should not be checked if it is only used as step in the perfusion procedure.
- **Perfusion**: Check the perfusate used (check all that apply). If not listed, check Other and provide the perfusate(s) used. If any extra procedures not part of the perfusion preparation are performed once anesthesia is initiated but before perfusion begins (e.g. tissue collection, imaging or measurements requiring the body cavity to be open, etc.), the procedure may need to be described as a non-survival surgery in the Appendix A- Surgical Procedures.
- **Vital Organ Removal (fresh, unfixed)**: Check if vital organ removal is the primary rather than secondary cause of death. Indicate the vital organ(s) to be removed.
- **Thoracotomy**: Check here if thoracotomy is the primary rather than secondary cause of death.
- **Other**: Check if using another type of physical method under anesthesia that is not listed and describe the specific procedure used.

  - **Captive Bolt**: Indicate the secondary method and any additional method(s) used for verification of euthanasia.
  - **Other**: Indicate any other physical method utilized without anesthesia and provide the secondary method and/or any additional method(s) used for verification of euthanasia.

B. Provide scientific justification if any of the euthanasia methods listed are not described as "acceptable" or "acceptable with conditions" (provided the conditions are met) by the AVMA Guidelines on Euthanasia, 2020.
   - Include specific information and justification as to why an acceptable method cannot be used. You must also provide a statement detailing the experience of the personnel that will be performing this euthanasia. You should ensure all relevant personnel are adequately trained and maintain any records that document training in the method(s) of euthanasia.
   - If you will be using an AVMA acceptable or acceptable with conditions method, list N/A in this section.

C. If some animals will not be euthanized at the end of a study, include a description of what will be done with the animals (e.g. transfer to another protocol, adoption, sold at market, release-wildlife studies only).
   - If animals will be transferred to another protocol, be sure to fill out the OLAR Transfer Form or other appropriate transfer mechanism prior to the transfer.
   - If animals will be adopted out, there is an Adoption Application form that you should complete and submit. The adoption must be approved by the Attending Veterinarian (or designee) PRIOR to the animal leaving WVU. Please see the IACUC Animal Adoption Policy for more information (https://animal.research.wvu.edu/policies-and-guidelines).
   - Wild animals that are to be returned to the wild require special consideration and prior approval by the IACUC.

10. POLICIES, PROCEDURES, AND GUIDELINES

PLEASE NOTE: IACUC policies are reviewed and revised periodically, therefore confirm that you are using the most current version of the protocol form. The most current version can be found on the OAW website: https://animal.research.wvu.edu/protocols

A. This section lists IACUC-approved Policies, Guidelines, and standard operating procedures (SOPs). You must check one of the three options, "YES", "NO", or "N/A", for EACH. All of these documents can be found at: https://animal.research.wvu.edu/policies-and-guidelines.
   - Check "YES" for each document that is applicable to your protocol.
   - If the document is applicable to your protocol but you cannot follow it or require an exception to certain aspects of the policy, guideline or SOP, check "NO". * Follow the instructions below, See C.
   - If the document is not applicable to your protocol, check "N/A".

B. This section lists IACUC-approved Core Facility SOPs. If your protocol involves any of these, you must follow the information stated in the SOP that pertains to your work. Procedures utilizing Core Facilities must still be described in a procedural appendix.

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.

D. If you have checked "YES" for any of the IACUC-approved policies, guidelines or SOPs, you must 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 cannot follow them as written. Contact the OAW Office.

11. REPLACEMENT, REDUCTION AND REFINEMENT OF USE OF ANIMALS

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, computer models, etc.
   2. Justify the species you will be using based on literature, previous studies, unique anatomy, 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/stress category D or E), this section is required.

Efforts must be made to improve procedures and methods to reduce animal numbers and morbidity or mortality.

1. Non-USDA-regulated species – Summarize why you cannot use alternatives for each painful or distressful procedure within this protocol.
2. USDA-regulated species – List/describe individual procedures that may cause pain or distress.
3. For USDA-covered species, 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. The search to be completed in this section must be a search for alternatives to potentially painful or distressful procedures. 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:
    - The Databases searched
    - The dates the search was completed
    - Publication date range searched
    - Boolean operators and search words that were used. Boolean operators are "Simple words used to combine or exclude keywords in a search, resulting in more focused and productive results. (e.g., 'and', 'or' and 'not').
    - The number of hits for each
4. You must indicate if alternatives to the procedures that you listed in section 11.C.1 were found.
5. 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 cannot be used to meet your study goals.

APPENDICES

APPENDIX A– Surgery and Management of Pain and Distress

If your project requires surgery of any kind, this appendix must be completed. Fill out a separate Appendix for EACH surgery. For each appendix, indicate the total number of animals that will undergo those procedures in the upper right section and the species in the upper left section. In Section 10 of the main form, be sure to check any of the applicable policies or guidelines related to surgical procedures for the species you will be using.

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. Check Minor or Major based on the definitions provided on the form.

1. Give the name of the surgical procedure, and for consistency, utilize the same name in the experimental design and appendix. Provide a detailed step by step description. Information provided should include (but is not limited to):
    - Anesthetics, analgesics, or tranquilizers that will be used and the timing for administration. Include specifics of the drugs in Appendix C-Drugs.
    - Skin preparation prior to incision.
    - Pre- and peri-operative care of animals, including how anesthetic depth is monitored.
    - Specific information regarding wound closure and, if applicable, suture type, pattern and timing of suture removal.

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

3. 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):
    - Intraoperative monitoring techniques.
    - Steps that will be taken to avoid or minimize pain or distress to animals.
    - Criteria used to determine when animals should be euthanized (i.e., humane endpoint criteria).

4. Give your best estimate regarding possible percentages for morbidity and/or mortality due to the proposed procedures. **Importantly, your response should cover not only the procedure itself but also the disease or infection model the procedure is meant to induce, if applicable**. This should include (but is not limited to):
    - A description explaining this percentage.
    - Likely causes for morbidity and/or mortality and how they will be addressed.

5. If an animal will undergo multiple survival surgeries, check "Yes" and below check whether it will be Multiple minor survival surgeries (with no major), One Major and one or more minor survival surgeries, or Multiple Major survival surgeries.

6. If Multiple Major was checked in #6, provide an explanation and scientific justification as to why multiple major surgical procedures are essential to your protocol. Also include the amount of time between surgeries, and if applicable, what additional steps will be taken to ensure the animal's health and well-being. Note: surgical procedures performed prior to the animal's arrival at WVU count toward the total of surgical procedures (e.g. ovariectomized mice).

APPENDIX B – Non-Surgical Procedures

Details of any non-surgical procedures should be included in this appendix. Fill out a separate Appendix B for EACH non-surgical procedure (or procedure type). If procedures involve prolonged restraint of unanesthetized animals or food/water changes, an Appendix H and Appendix S, respectively, must also be filled out.

1. Identify each non-surgical procedure by name. For consistency, utilize the same name in the design and appendix.

2. Provide specific details of the non-surgical procedure. Be sure to include the purpose, duration, frequency, and any special monitoring and removal criteria, if applicable.
    - 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.

3. Check Yes or No as to whether the non-surgical procedure involves the use of anesthesia, If yes, you must describe the anesthesia induction, monitoring, and post-procedural care. Information should include (but is not limited to):
    - The anesthetic used (also include in Appendix C-Drugs).
    - How depth of anesthesia will be determined.
    - The frequency (during anesthesia and recovery) and duration of monitoring.
    - Analgesics given, if applicable (also include in Appendix C-Drugs).
    - Criteria for determining that the animal has recovered.

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 any potential adverse effects. **Importantly, your response should cover not only the procedure itself, but also, when applicable, any outcomes related to the purpose of the procedure. For example, the effects of the experimental substance(s)**

administered, the induced disease or infection model, the induced physiological or behavioral state, etc.

APPENDIX C – Use of Drugs in Animals (non-euthanizing agents)

1. List in this appendix all anesthetics, analgesics, sedatives/tranquilizers, and experimental drugs and chemicals that will be used for the experimental procedures described. Indicate the species in top left. Include in the chart for each substance used:
    - Dose or dosage range in mg/kg.
    - Route of administration (e.g., IP, SC, IM, inhalation, oral, etc.).
    - Frequency (how often, e.g. once, daily, weekly, etc.).
    - Number of doses (range or maximum).
    - Procedures each substance is used for.
    - For Other/Experimental Drugs or Chemicals, manufacturer and catalog number must be included for the purposes of the Chemical Safety review.
        - Biological substances administered to animals should also be listed in this section (e.g. biological toxins, bacteria, cells, viral vectors, etc.).

Hazardous Chemicals

It is the PI's responsibility, in consultation with the Environmental Health and Safety (EH&S), to determine if any drug/chemical is considered hazardous. It is also the PI's responsibility to provide hazard-related health and safety information to personnel on the protocol that will be working with the hazard(s). Chemicals or drugs that are considered hazardous may require a Standard Operating Procedure (SOP) be submitted and approved by EH&S. You may contact EH&S for assistance, and note that all IACUC protocols undergo a Chemical Safety review. If hazardous chemicals are being used, the Yes box should be checked in Section 10.A Approved IACUC Policies, Guidelines, and SOPs of the main form.

Non-Pharmaceutical Grade Compounds

If you are using non-pharmaceutical grade compounds, please review the IACUC Policy "Non-Pharmaceutical Grade Substances Used in Animals" and provide a scientific justification. See the policy for definitions of what the IACUC considers pharmaceutical grade and examples of acceptable justifications. This information can be provided underneath the chart or in Section 10.D of the main form. The PI must also provide any available information on grade, purity, etc. and describe how the substance is compounded prior to administration to ensure proper pH and sterility, to the extent possible. Unless otherwise described in a relevant procedural appendix, this additional information can be provided underneath the chart with the non-pharm justification.

APPENDIX E – Breeding Animals

This appendix must be completed if animal breeding is listed in your approved IACUC protocol. Note that this appendix will pertain mostly to rodent breeding. If you will be breeding other species (e.g., sheep, cattle, etc.), indicate N/A for those questions that do not apply.

Describe your breeding plan by answering all of the questions.

1. Indicate the type of breeding that 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 postpartum 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 – Your AI procedure should be described in either an Appendix A or B if it is surgical or non-surgical, respectively.
    - Other: Describe your other breeding method
        - Rats and Mice: If you cannot follow the IACUC policy "Breeding and Weaning of Rats and Mice", describe your breeding strategies here as an exception to the IACUC Policy.

2. Estimate how many breeding animals will be used for each strain to maintain the line of animals. It would be helpful to indicate the number of males versus females, especially if you will be using the harem breeding strategy.

3. Indicate how many litters or offspring will be produced based on the number of breedings and strains as well as the anticipated average number of pups per litter. Keep in mind that not all breeding setups may be successful.

4. Indicate approximately how many or what percentage of litters or offspring will be used for experimental purposes.

5. Indicate approximately how many or what percentage of offspring will be unusable (i.e., incorrect genotype, etc.). Offspring that are euthanized because they cannot be used experimentally or transferred to other protocols should be listed as Category C in Section 5. of the main form.

APPENDIX F – Genetically Modified Animals

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

Note: The creation of genetically modified animals requires approval from the Institutional

Biosafety Committee (IBC), and Appendix I must also be completed.

1. List all known genetically modified strains of animals that will be used on this protocol. If additional genetically modified strains are needed at a later time, the protocol must be amended to include those.

2. If you are using the Transgenic Animal Core Facility (TACF) to generate genetically modified animals as part of this protocol, you must:
    - Get approval from the IBC.
    - Have the TACF 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 TACF). This may be done subsequent to approval of your protocol, or in tandem.

3. If you will not be creating NEW genetically modified animals at WVU, indicate the source (e.g. internal collaborator, other institution or vendor) of the GMAs that you will be using.

4. Indicate if you will be breeding GMAs, and if Yes, complete Appendix E.

5. When using GMAs, provide a plan as to how you will monitor intended and unintended phenotypes, especially those that have the potential to cause pain or distress.
    - How often will the animals be handled and/or observed?
    - What signs of pain or distress will you watch for?
    - Is any special care needed for the animals (e.g. sterile housing conditions, soft bedding, etc.)?
    - If pain or distress does develop, what will your plan be for management of that pain or distress? What are your humane 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.

APPENDIX G – Pain Category E Justification

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).

1. Describe the scientific justification for why pain or distress cannot be relieved, and the reason why other procedures that do not cause pain or distress 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 (frequency, responsible individual, signs, etc.).

3. It is the IACUC's legal requirement to evaluate the amount of the potential harm caused to the animals in relation to the benefit of the research to humans, animals and/or basic research. Explain why the pain/distress to which the animals will be subject is worth the benefit to human health, animal health, society, or advancement of science.

4. Experimental (Research) endpoint is defined as the point at which the scientific aims and objectives have been reached (Guide, 8th ed.). Indicate the research endpoints of the study.

5. Humane (Clinical) end point is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved (Guide, 8th ed.). Indicate the humane endpoints that will be used to determine when an animal must be removed from a study.

APPENDIX H – Prolonged Physical Restraint

If any prolonged physical restraint (≥ 30 minutes in a natural position or ≥10 minutes in an unnatural position) is required for your protocol in animals not under general anesthesia, you must complete this appendix. Prolonged restraint should be avoided, if possible. This appendix is not to be used for animals that undergo momentary restraint for the purposes of procedures such as IP injections, ear tagging, tail vein injections, vaccinations, shearing, shaving, etc.

Please see the IACUC Policy "Prolonged Physical Restraint of Animals". Also, if applicable, make sure to check where appropriate in sections 5.A (check yes to "Prolonged Restraint") and 10.A. of the main form.

1. State the number of times and the frequency of restraint for each group (e.g., once a week for five weeks). If some groups of animals will be restrained more times than other groups, be sure to make this distinction.

2. Indicate the duration the animals will be restrained during each restraint period.

3. Describe how you plan to acclimate the animals to the restraint device. Include what steps are taken for animals that do not adapt well to restraint. If animals will not be acclimated to restraint, please justify.

4. Describe the restraint device, the steps required to restrain the animals, and the plan for monitoring the animals while they are being restrained.

5. Describe how the stress of restraint will be kept to a minimum. Include criteria used to determine an animal needs to be removed early from restraint. If stress cannot be minimized, a justification must be included.

APPENDIX I – Use of Biohazardous or Infectious Materials in Animals

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

a. Provide the status of the Institutional Biosafety Committee (BC) protocol submission, if applicable. Please consult with the Biosafety Officer for the IBC to determine if IBC approval is required. If IBC approval is required, final approval of IACUC protocols will be held until receipt of the 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).

b. Specify pathogen and host species.

c. Indicate the method of infection or administration of the biohazard or infectious material.

d. Indicate the length of time animals are maintained after exposure or infection.

e. Provide the pathogen-specific Standard Operating Procedure (SOP) that will be followed for use in the animal facility. A separate standard operating procedure (SOP) must be included if the "OLAR ABSL-2 Use in Animals" does not apply to the biological material you will be using. Please consult with OLAR for further guidance.

APPENDIX J – Use of Radioactive Materials in Animals

This appendix must be completed if any radioactive materials are to be used in animals. Refer to the IACUC guideline "Guidance for Use of Radioactive Materials in Animal Studies" (https://animal.research.wvu.edu/policies-and-guidelines) and visit the website of the Radiation Safety Office for more information: https://www.hsc.wvu.edu/rsafety.

a. If Radiation Safety Committee (RSC) approval is required, final approval of the IACUC protocol may be held until receipt of the RSC approval.

b. Provide the name of the Authorized Radiation User (ARU)

c. Specify the nuclide and how it is used.

d. Indicate the route of administration.

e. Indicate the duration of exposure.

f. Provide the length of time animals are maintained after exposure.

g. Provide the location of exposure, including any room(s) in which animals are temporarily held until no longer radioactive. This should also be listed in Section 6.B. of the main form.

h. Provide the nuclide-specific SOP that will be followed for use if in the animal facility, if applicable.

APPENDIX K – Satellite Housing Request

If animals must be housed for >24 hours (or >12 hours for USDA-covered species) outside of the animal facilities, complete this appendix. Prior to housing animals in a satellite facility, a physical inspection of the space and IACUC approval is required. All provisions regarding animal husbandry and environmental conditions as well as veterinary care outlined in both the Guide and Animal Welfare Regulations (AWRs) should be adhered to at the satellite facility, as is required in the main animal facilities.

At the top, designate the species and maximum number of animals and cages/tanks/pens.

1. Provide scientific justification for satellite housing relative to your research, teaching, maintenance, or service needs on the specific protocol (note that justification cannot include cost or convenience).

2. Give the specific location of the proposed satellite facility and provide information regarding security of the room (e.g. hard key, key card, biometric entrance system). Include the appropriate type of housing (microenvironment), which may include the following: static rodent caging or tanks, ventilated caging, recirculating systems, pens, bird cages, etc.

3. List all personnel who will be responsible for daily monitoring of environmental conditions. List the method of how you will record the daily data. If you are unsure about how or what to monitor, contact OLAR for assistance.

4. Indicate the frequency in which the microenvironment will be changed/cleaned. Specify how you plan to provide and monitor the fresh food and water needs of the species in the satellite. List all personnel that will be responsible for husbandry in the satellite. Husbandry duties can include the following:
    - Who will be caring for the animals;
    - Bedding, water and cage change schedule; and
    - 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 animal health checks, cage changes, provision of fresh water and food, temperature, humidity, etc. If you need a sample log, please contact OLAR.

5. It is imperative that there be emergency contact information of personnel provided and posted in case of an emergency. Indicate multiple methods for contacting these individuals (cell phone number, home number, email, etc.). Providing more than one emergency contact is strongly recommended.

APPENDIX L – Progress Report for Protocol Renewal

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 the work that has been performed on the animals for this project in the past 3 years. This should include the completed aims from previous 3 years of the original protocol.
- The number of animals that have been used for this project so far.
- Optional – a short list of publications, poster abstracts, or grants that have come from the work on the current protocol (within the last three years).

APPENDIX N: Additional Species

This appendix needs to be completed if you plan to use more than 2 species of animals in your protocol (e.g., mice, rabbits, cows and salamanders). The information requested is the same as in Section 5 "Animal Information", in the main protocol form (see instructions for this section).

You may choose to use the charts in this appendix to indicate separate phenotypes of the same species that may cause pain and distress.

Although this is not necessary, you may also choose to list the strains separately to indicate additional groups of the same species.

APPENDIX O: Single Housing

Social Housing is defined as housing social species in compatible pairs or groups with additional visual, auditory, olfactory, and/or tactile contact of conspecifics housed within the same room. For further information, see the IACUC policy "Social Housing of Research Animals" (https://animal.research.wvu.edu/policies-and-guidelines).

This appendix should be completed if you plan to singly house your animals for any length of time, and your reason for requiring single housing is not included as a program-wide approved IACUC exception in the social housing policy referenced above.

1. Indicate whether any of your animals will be singly housed.

2. Indicate the reason the animals will be singly housed and provide a scientific justification.

3. Single housing should be limited to the minimum period necessary to accomplish the study goals. How long will the animals be singly housed?

4. Indicate if any singly-housed animals may be returned to social housing at the end of the study or at any point in time during the study.

5. If "no" was checked for item 4, justify why animals cannot be returned to social housing as per the Guide (8th Ed.).

If you have any questions or concerns regarding the information requested in this appendix, please contact a member of the OLAR veterinary staff or OAW.

APPENDIX R: Field Work

For protocols involving field work, this appendix needs to be completed in order to identify potential personnel safety or pathogen transfer concerns, impacts of habitat manipulation, effects on non-target animals, and additionally to provide information on animal transport if applicable.

1. Describe the location of your study area and include any safety concerns that personnel should be aware of related to the area and/or procedures utilized (e.g. electrofishing).

2. If any transport of live animals takes place, describe the types of container(s) that may be utilized, transport method (by vehicle, etc.), and methods to secure animals during transport.

3. Describe any manipulation of the habitat, including any equipment or materials utilized, that may intentionally or unintentionally disrupt the natural habitat of target or non-target species.

4. Describe methods and procedures for limiting transfer of pathogens between subjects, species, or colonies/animal groups. Include any needed decontamination of equipment and PPE, where applicable.

5. Indicate any effects of your field work activities on any non-target animals. List the species that may be affected and any plans to mitigate effects if applicable. If any non-target animals that may be affected are considered protected or endangered species, please indicate.

APPENDIX S: Food/ Water Changes for Study Purposes

This appendix covers food and water changes that fall under the provided definitions of manipulation, deprivation, regulation, and/or restriction. Please indicate the species if there is

more than one species utilized on the protocol.

1. Select all applicable food and water changes. Note that a description of these changes should be described in a relevant procedural appendix or in a stand-alone appendix, as appropriate. For example, fasting for glucose measurements can be included in the appendix describing the glucose testing procedure. Food regulation or restriction that is a part of a specific behavior test can be included in the relevant behavior appendix or a stand-alone appendix if it is more broadly utilized for a behavioral protocol (i.e. not tied to a specific procedure).

2. Indicate any possible adverse consequences that may result from the food and/or water changes and what steps will be taken to prevent or mitigate those effects. If adverse consequences are not anticipated due to previous experience, literature support, etc., please specify.

3. Indicate how animals will be monitored during the food and/or water changes and what observations or criteria would be used to temporarily or permanent remove the animals from food and/or water control.

4. Indicate how animals will be fed and/or watered on days where change for study purposes is not needed. If they are not returned to normal husbandry nor maintained on the change throughout the duration of the study, please select Other and describe.