# **Instructions for completion of Notice of Intent Form**

1. Answer all questions and sign the Investigator Assurance. Incomplete forms will be returned without review.
2. Please use a minimum of 11 point font. Handwritten forms will be returned without review.
3. All questions on the Protocol Form must be responded to directly and completely. The Committee will **not** review forms with answers that are not responsive to the questions. The Committee will **not** review responses that refer to “see attached” portions, consisting of portions of the grant/contract applications, dissertation proposals, or other material prepared for another purpose.

NOTE: The Protocol Forms are designed to provide the Committee, when properly completed, with only that information that is required for the Committee to make a proper and valid evaluation.

1. Please state in language that can be understood by a non-scientist (layperson, for example, a high school senior) or state as you would for a newspaper article. This means minimal use of technical terms and a brief explanation of those terms you must use. Please avoid the use of scientific jargon, acronyms, and abbreviations. To check for this please visit <https://www.webfx.com/tools/read-able/> . Do not cut and paste from the grant application.

# **Instructions for Searching for Alternatives**

The search for alternatives refers to the three Rs described in the book *The Principles of Humane Experimental Technique* (1959) by Russell and Burch. The 3Rs are reduction in the number of animals used, refinement of techniques and procedures to reduce pain or distress, and replacement of animals with non-animal techniques or use of less-sentient species.

**Refinement:** The use of analgesics and analgesia, the use of remote telemetry to increase quality and quantity of data gathered, and humane endpoints for the animals are examples of refinements.

**Reduction:** The use of shared control groups, preliminary screening in non-animal systems, innovative statistical packages or a consultation with a statistician are examples of reduction alternatives.

**Replacement:** Alternatives such as in vitro, cell culture, tissue culture, models, simulations, etc are examples of replacement. This is also where you might look for any non-mammalian animal models – fish or invertebrates, for example – that would still give you the data you need.

**The AWIC (Animal Welfare Information Center) recommends alternative searches be performed in two phases.** Phase 1 considers reduction and refinement and the recommendation is NOT to use the word “alternative” unless the particular area of research happens to be an area in which there has been considerable work in developing alternatives (e.g. Toxicology and education). This phase should address duplication, appropriate animal numbers, the best pain-relieving agents and other methods that serve to minimize or limit pain and distress.

Phase 2 of the search is focused on Replacement. In this phase, the use of the work “alternative” is appropriate and the use of the word “model” is recommended. The result of this second phase of the search is supposed to retrieve information on animal and non-animal models as potential alternatives.

The search strategy consists of the reduction and refinement phase and the replacement phase as mentioned before. The reduction and refinement phase should be similar to the typical literature review done in preparation for a new project or scientific publication. Keywords used will help the researcher determine if there is unintentional duplication, how many animals are necessary using the proposed model, appropriate anesthetics and analgesics, and any other method of minimizing pain and distress. Since much of the refinement and reduction information will be found in the materials and methods sections, it is important for the researcher to review some of the articles that may be of interest.

Many people make the mistake of putting the term “alternatives” into the strategy and expect to find all possible alternatives. Because “alternatives” is a complex concept involving refinement, reduction, and replacement, this term is best used only in those areas of study where larger amounts of research have been conducted on alternatives, such as in toxicology or education.

The replacement phase should include keywords for potential alternatives such as “vitro”, “culture”, or “simulation”. The word “alternative” may also be included here. The selected animal model, other species, and the word “model” will help retrieve animal and non-animal models as potential alternatives.

| **Leave Blank**  Protocol#:  Expiration Date: |
| --- |

East Tennessee State University

Animal Study Protocol

| For UCAC only: Disposition:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  UCAC Chair (signature), date |
| --- |

Version 7/2022

Please type.

# **General Information**

Principal Investigator/Course Director:

Degree(s):

Academic Title:

Department:

Campus Address:

Email:

Phone:

Emergency contact name and phone:

Department administrator, phone:

Grant/Project Title:

# **Sponsorship of Project**

Has funding for this project been applied for? Yes No

If yes, please attach copies of grant pages or project summary describing animal care and use.

Funding agency/source of funds:

External grant/Contract No. (if known):

Inclusive dates of project funding: From:       To:

Will research start before the grant? No Yes

If yes, give start date:       Alternate source of funds:

# **Type of Proposal (check one)**

New Protocol

Renewal of Protocol #

Modification of Protocol #

(Submit most recently approved version of your protocol in its entirety with new material highlighted. Also include cover letter describing which Statements have been modified.)

# **Procedure Classification Categories**

| B. Experiments which involve no pain, distress, or use of pain-relieving drugs (e.g. breeding) | D. Experiments which may potentially cause pain or distress to animals and for which appropriate anesthetics, analgesics, or sedatives are used (submit Statement C) |
| --- | --- |
| C. Experiments which may involve momentary or slight pain or distress, including short-term physical restraint, injection, blood sample from a peripheral vein, or euthanasia (submit Statement C) | E. Experiments involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or sedative drugs would adversely affect the procedures, results, or interpretation of the experiments (submit Statement E) |

# **Number of Animals**

Specify the number of animals to be used (experimental and breeding) **for the entire 3-year project**:

| Species | Type B | Type C | Type D | Type E | Totals\* |
| --- | --- | --- | --- | --- | --- |
| 1. |  |  |  |  |  |
| 2. |  |  |  |  |  |
| 3. |  |  |  |  |  |
| 4. |  |  |  |  |  |

*\*Number here must agree with the number scientifically justified in Section V. C. below*

# **Justification**

1. **What is the objective of these experiments?**
2. **Harm-benefit analysis.** 
   1. **What harm impact on the 5 freedoms (freedom from pain/injury, fear/distress, hunger/thirst; ability to express normal behavior and freedom from discomfort) will the animals experience?**
   2. **In what way will the results be beneficial to society?**
3. **Why is it necessary to use this species for this purpose?** *Please explain why a species phylogenetically lower cannot be used*

**Provide scientific justification for the number of experimental and breeding animals proposed for this study.** *Explain how the number of animals requested will be utilized. Please briefly detail the experimental design so that the reviewer(s) can ascertain how the number of animals requested are used. Include a table or flowchart as appropriate. Please include a brief description of the statistical analyses, including tests, power and probability levels utilized, if applicable. Please note: “time” (number of animals per day, month, or experiment) is not considered to be scientific justification. For the breeding animals be sure to account for those animals which are the “incorrect” genotype and which will not be used as well as the replacement of breeders. Number here must agree with the number in table under Section IV.*

1. **Method of identification of animals:** Identification is required for all animals; rodents and smaller animals need not be identified individually. Check all that apply.

cage card  ear punch tattoo

other (specify):

# **Personnel**

Provide the following information for all personnel to be involved with this study that will have contact with animals or their tissues, including entering the animal facility: *This includes the Principal Investigator, co-investigators, technicians, students, etc. All personnel involved with this project must have completed the Online Research Training Program* ([ETSU DLAR Animal Use Training](http://www.etsu.edu/com/dlar/training.php) ).

| Name: | PI: |  |  |
| --- | --- | --- | --- |
| Experience/training with all animal species listed | No Yes:  How long? | No Yes:  How long? | No Yes:  How long? |
| Experience with all procedures performed on these animals | No Yes:  How long? | No Yes:  How long? | No Yes:  How long? |
| Describe the animal-related responsibilities for each person |  |  |  |
| Completed Online Research Training Program: | No Yes:  Date: | No Yes:  Date: | No Yes:  Date: |
| Current Risk Inventory Form for this laboratory on file with the OHO (annual updates required) | No Yes:  Date: |  |  |
| OHP Health Assessment form submitted to OHO (annual updates required) | No Yes:  Date: | No Yes:  Date: | No Yes:  Date: |

If your answer to any of the above questions is “No”, please explain:

\*for additional personnel, please copy the above table.

# **Animal Facility**

Where will the animals be housed?

VA 119 Brown Hall Other (Complete Statement A for Satellite Facility)

Room Number:

Will the animals be removed from the DLAR?

Yes No

If Yes, to: Bldg       , room#

Will these animals be returned to DLAR?

Yes No

If yes, to: DLAR bldg.:      , room #

*(Note – Return of any animals to DLAR facilities requires special housing arrangements)*

Will any animals be moved off the ETSU/VA Campus?

Yes No

If Yes, give reason(s), destination, and means of transportation:

# **Project Details**

Check box if applicable:

Yes Will animals be housed longer than 11.5 hrs (USDA covered species) or 23.5 hours (non-USDA covered species) in facilities other than those listed above?

*IF YES*, attach a **STATEMENT A: Satellite Animal Facility**

Yes Does the research protocol require deviation from standard water/diet, diet restrictions, or other than normal environmental conditions?

*(Including single housing of social species and use of wire-bottom cages)*

*IF YES*, attach a **STATEMENT D: Diet Manipulation and Environmental Modifications**

Yes Will you be collecting body fluids from animals, other than fluid collection just prior to euthanasia?

*IF YES*, attach a **STATEMENT F: Antemortem Fluid/Tissue/Tail Collection**

Yes Will any animals be euthanized during or after this study?

*IF YES*, attach a **STATEMENT G: Euthanasia**

Yes Will non-survival\* surgery be performed on the animals?

*\*animal will be euthanized at the end of the procedure without recovery (e.g., terminal perfusion of anesthetized animals)*

*IF YES*, attach a **STATEMENT H: Non-Survival Surgery**

Yes Will survival\* surgery be performed on the animals?

*\*survival is defined as recovery from anesthesia*

*IF YES*, attach a **STATEMENT I: Survival Surgery, Analgesics and Antibiotics**

Yes Does this study require anesthesia, analgesia, sedation, or tranquilization of animals?

*IF YES*, attach a **STATEMENT K: Anesthesia, Sedation, or Tranquilization**

Yes Does the protocol require learning, behavioral, or memory screening of animals?

*(These include locomotor activity, Y-maze, operant conditioning, etc)*

*IF YES*, attach a **STATEMENT L: Behavioral Screening/Conditioning**

Yes Will you be using animals to produce either monoclonal or polyclonal antibodies?

*IF YES*, attach a **STATEMENT M: Antibody Production and Vaccine Challenge**

Yes Does the research protocol involve the use of genetically modified strain(s) in which the alteration is known or suspected to influence morbidity and/or mortality?

*IF YES*, attach a **STATEMENT P: Clinically Adverse Rodent Phenotype**

Yes Will any animal be restrained\* in a manner other than caged during this study?

\*t*his does not include momentary restraint for simple procedures (i.e. injections)*

*IF YES*, attach a **STATEMENT R: Animal Restraint**

Yes Does the research protocol involve the use of infectious agents, hazardous chemicals, genetically altered materials, recombinant DNA or radioactive materials?

*IF YES*, attach a **STATEMENT S: Biohazard and Special Requirements**

Yes Does this project involve the production or study of tumors in animals?

*IF YES*, attach a **STATEMENT T: Tumors in Animals**

Yes Will exogenous substances or tissue (e.g. drugs, infectious agents, carcinogens, toxins, etc) be administered to animals other than for anesthesia or euthanasia?

*IF YES*, attach a **STATEMENT X: Exogenous Substance Use**

Yes Does this project involve field/wild animal studies?

*IF YES*, attach a **STATEMENT Y: Field/Wild Animal**

Yes Other Procedures. Are other procedures planned but not described elsewhere in the Notice of Intent?

*IF YES*, attach a **STATEMENT Z: Other Procedures**

No Are all personnel (faculty, students, and staff) having contact with the animals in this

Yes project participating in the Occupational Health and Safety Program as recommended

by the University Committee on Animal Care? ([UCAC OH&SP](http://www.etsu.edu/ucac/occupationalhealth.php) )

No Will a copy of this protocol be provided and read by the personnel conducting the

Yes procedures?

No Have alternatives to the use of vertebrate animals been considered?

Yes

No Are there procedures in the protocol which are not contained in the attached grant

Yes pages? *If so, procedures in the protocol supersede grant pages.*

# **Investigator Assurance**

1. I certify that I have completed the Online Research Training Program.
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this protocol and who are exposed to animals or their viable tissues or waste, are participating in the ETSU Occupational Health and Safety Program.
4. I certify that only the individuals listed in Sections VI are authorized to conduct procedures involving animals under this protocol, have completed the Online Research Training Program, and have received training in: 1) the biology, handling, and care of the species to be used; 2) aseptic surgical methods and techniques (if necessary); 3) the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; 4) the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and 5) procedures for reporting animal welfare concerns.
5. For all Pain/Distress Classifications C, D, and E proposals, I certify that I have reviewed the pertinent scientific literature and the sources and/or databases and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
6. I certify that I will obtain approval from the UCAC before initiating any significant changes in this study; this includes substantive changes in the procedures, changes in personnel performing the procedures on animals, or in the number of animals to be used.
7. I certify that I will notify the UCAC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity or mortality, will be reported to the Attending Veterinarian and the UCAC.
8. I certify that I am familiar with and will comply with all pertinent institutional, state, and federal rules and policies.

| \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| --- | --- |
| Signature of Principal Investigator | Signature of Department Chair |

## Statement A: Satellite Animal Facility

*(If this statement is blank, omit it)*

All spaces where animals are held longer than **11.5 hours (USDA covered species) or 23.5 hours (non-USDA covered species)** must meet legal requirements for the housing of animals and be approved by the UCAC. **Please call the UCAC office at 439-6292 for details.**

1. Give location:

Bldg       Room #

1. Give a justification for this location:
2. Will investigator be responsible for husbandry of animals while housed in this facility? Yes No

If yes, has an UCAC approved SOP been provided and reviewed with all personnel? Yes No

1. Has this location been inspected by the UCAC within the last six months? Yes No

## Statement C: Justification for Type C/D Animal Use

*(If this statement is blank, omit it)*

1. Species:

*Procedures that may cause more than momentary or slight pain or distress* ***must****, in their planning, involve consultation with a veterinarian trained in Laboratory Animal Medicine. Dr. Greg Hanley may be reached by calling the Division of Laboratory Animal Resources at 439-6783.*

1. Which veterinarian have you consulted?

Date consulted:

1. List procedures that could potentially cause pain or distress that you propose to use:
2. Alternatives to painful or distressful procedures:

Refer to the Instructions for Searching for Alternatives at the beginning of this form.

| **The minimal written narrative must include:**   * **Databases searched** or other sources consulted * **Date of the search** * **Years covered by the search** * **Key words or search strategy** used by the Principal Investigator when considering *alternatives* to the above listed procedures or descriptions of other methods. **This information should provide assurance that there are no alternatives available to the painful or distressful procedures listed above.** The Narrative should be such that the University Committee on Animal Care can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough. The potentially painful/distressful procedures must be included as a keyword, as well as the species and the word “alternative”. |
| --- |

Please provide your narrative here:

1. Results of search:

No alternatives were found

Yes, alternatives were found. Explain why they cannot be used:

## Statement D: Diet Manipulation and Environmental Modifications

*(If this statement is blank, omit it)*

*Attach a* ***separate*** *Statement D for each species*

1. Species:
2. Describe how dietary/water manipulations or restrictions will be accomplished:
3. How long will the animal be maintained in this nutritional state?
4. Physical/Physiological effect:

Will physical or physiological effects(s) (e.g. low levels of vitamin C result in Scurvy, low

levels of calcium result in bone abnormalities, and/or weight loss, etc) likely result from

this treatment? No Yes

*IF YES*, describe, in detail, including criteria for termination of the experiment:

1. Environmental alterations:

Will the protocol require an alteration in the standard lighting (12 hrs on – 12 hrs off),

temperature, or other environmental variables? No Yes

*IF YES*, describe, in detail:

1. The Guide for the Care and Use of Laboratory Animals states that social species should be housed in pairs or groups unless experimental, health, and behavioral reasons, e.g., fighting, might preclude a successful outcome of this kind of housing.

Will social animals be housed singly? No Yes

*IF YES*, include single housing in Statement C and provide scientific justification here:

1. The Guide for the Care and Use of Laboratory Animals recommends the use of contact bedding (i.e., shoebox or microisolator cages) instead of wire mesh floors (e.g., metabolism cages) for housing rodents.

Will the animals be housed in wire—bottom cages? No Yes

*IF YES*, include wire-bottom cages in Statement C and provide scientific justification here:

## Statement E: Justification for Type E Animal Use

*(If this statement is blank, omit it)*

1. Species:

*Procedures that may cause more than momentary or slight pain or distress* ***must****, in their planning, involve consultation with a veterinarian trained in Laboratory Animal Medicine. Dr. Greg Hanley may be reached by calling the Division of Laboratory Animal Resources at 439-6783.*

1. Which veterinarian have you consulted?

Date consulted:

1. List procedures that could potentially cause pain or distress that you propose to use:
2. Alternatives to painful or distressful procedures:

Refer to the Instructions for Searching for Alternatives at the beginning of this form.

| **The minimal written narrative must include:**   * **Databases searched** or other sources consulted * **Date of the search** * **Years covered by the search** * **Key words or search strategy** used by the Principal Investigator when considering *alternatives* to the above listed procedures or descriptions of other methods. **This information should provide assurance that there are no alternatives available to the painful or distressful procedures listed above.** The Narrative should be such that the University Committee on Animal Care can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough. The potentially painful/distressful procedures must be included as a keyword, as well as the species and the word “alternative”. |
| --- |

Please provide your narrative here:

1. Results of search:

No alternatives were found

Yes, alternatives were found. Explain why they cannot be used:

1. Justify no pain relief:

*Provide a scientific justification for why drugs, which might alleviate pain or distress, will be withheld. Document the rationale for this decision and provide references, if possible. (Euthanasia employed prior to significant pain or distress would not be Type E.)*

Expected clinical signs of pain/distress:

*Please describe clinical signs to be expected. Indicate the severity and duration of each clinical sign, the frequency the animal will be monitored, and when the pain will be eliminated or managed (euthanasia, drugs, or withdrawal of painful stimulus.) The committee must understand that the pain is the minimum needed for the shortest time possible, consistent with the experimental goals.*

## Statement F: Antemortem Fluid/Tissue/Tail Collection

**Fluid/Tissue/Tail Collection Prior to Death (blood, urine, bile, lymph, tail, etc.)**

*(If this statement is blank, omit it)*

Attach a **separate** Statement F for each species

1. Species:
2. Fluid/Tissue/Tail:
3. Volume/Amount per collection:
4. Frequency of collection:
5. Total number of collections:
6. Method/Route/Technique of collection:
7. Anesthesia/Sedation:

Will the animals be anesthetized or sedated during the procedure? No Yes

*IF YES*, then attach a **Statement K: Anesthesia, Sedation, Tranquilization**

1. Mouse Genotyping

Will the UCAC-approved [Policy for Tail-Cutting in Rodents](http://www.etsu.edu/ucac/7.-policy-for-tail-cutting-of-rodents.docx) be followed?

No Yes N/A

[Safe bleeding volume for common laboratory species may be found in the *Formulary for Laboratory Animals, 2nd Edition*, Iowa State University Press, Ames, Iowa.

Maximum volume in mL/kg: cat-7.7; chicken-9.9; guinea pig-7.7; hamster-5.5; monkey (macaque)-6.6; mouse-7.7; pig-6.6; rabbit-7.7; rat-5.5; sheep-6.6]

## Statement G: Euthanasia

*(If this statement is blank, omit it)*

Attach a **separate** Statement G for each species

1. Species:
2. Chemical methods:

Carbon dioxide (see UCAC Policy on Use of Carbon Dioxide for Euthanasia)

Anesthetic agent overdose

If using an anesthetic agent, provide the following information:

Drug:

Dosage:

Route of administration:

USP/NF?\*\* No Yes

1. Physical methods:

Exsanguination (please list anesthetic agent above; if opening a body cavity, i.e., perfusion, complete Statements H and K.)

Cervical dislocation under anesthesia (please list anesthetic agent above)

Decapitation under anesthesia (please list anesthetic agent above)

\*Cervical dislocation without anesthesia

Provide Scientific Justification here:

\*Decapitation without anesthesia

Provide Scientific Justification here:

1. Other methods (describe in detail below):

\*The UCAC requires that a scientific justification be provided and approved for cervical dislocation or decapitation without prior sedation.

Rat or mouse feti may be humanely euthanatized by decapitation without anesthesia. The dam should be euthanatized as would any other adult animal. Please refer to the UCAC Policy on Euthanasia of Rodent Fetuses and Neonates.

\*\**USP/NF (United States Pharmacopeia National Formulary) refers to the formulation of the substance. For example, various substances can be purchased as chemical grade (non-pharmaceutical) form sources such as Sigma/Aldrich/Fluka. USP/NF grade substances are provided in a formulation specifically made for injection.*

## Statement H: Non-Survival Surgery

**(The animal is euthanatized at the end of the procedure without recovery from anesthesia)**

*(If this statement is blank, omit it)*

Attach a **separate** Statement H for each species

1. Species:
2. Which veterinarian have you consulted?

Date consulted:

1. Describe the surgical procedure(s) in detail:
   1. Aseptic preparation (at a minimum, the surgical site should be clipped, as required by The Guide):
   2. Procedure (include a description of the access to anatomic site):
   3. Expected duration of procedures:
2. Where will the surgery be performed:

Building:       Room #:

1. Who will perform the surgery?
2. Pre-operative care will include:

Withholding food: No Yes *IF YES*, for how long?

Withholding water: No Yes *IF YES*, for how long?

## Statement I: Survival Surgery, Analgesics and Antibiotics

**(The animal will recover from anesthesia)**

*(If this statement is blank, omit it)*

Attach a **separate** Statement I for each species

1. Species:
2. Which veterinarian have you consulted?

Date consulted:

1. Multiple procedures:

Will more than one survival surgery be performed on any animal?

No Yes *IF YES*, provide scientific justification:

1. Describe the surgical procedure(s) in detail:
   1. Aseptic preparation:
   2. Procedure (*include a description of the access to the anatomic site and the closure details. Use non-wicking sutures for skin closure; wound clips or sutures should be removed 8-10 days post-surgery*):
2. Where will the surgery be performed?

Building:       Room #:

1. Who will perform the surgery?
2. Pre-operative care:

Will food be withheld? No Yes *IF YES*, how long:

Will water be withheld? No Yes *IF YES*, how long:

1. Aseptic technique: *Aseptic technique includes wearing sterile surgical gloves, mask, and other surgical attire, as well as the use of sterile instruments and aseptic preparation of the surgical site.*

Will aseptic technique be used? Yes No *IF NO*, provide scientific justification:

1. Post-operative care: *Monitoring should be continuous until the animal has regained the ability to ambulate.*

Observation – Frequency:

Analgesics\*:

Drug:

Dosage:

Route:

Frequency:

The analgesic described above will be of USP/NF formulation.\*\* No Yes

Clinical symptoms which will result in administration of (additional) analgesics:

Antibiotics:

Drug:

Dosage:

Route:

Frequency:

The antibiotic described above will be of USP/NF formulation.\*\* No Yes

IF NO, provide scientific justification\*\*\*:

1. Other supportive care: *Describe in detail, and specify any fluids or special diet.*

1. Who will provide post-operative care:
2. Physical/Physiological effect(s):
   1. What complications, if any, ***may*** occur as a result of this surgical procedure (i.e. hemorrhage, wound infection, physical impairment, etc)?
   2. Describe in detail how complications will be managed and the criteria§ (i.e. clinical signs) which would result in termination of the experiment:
3. How long will the animal be maintained following surgery?

\**If pre-emptive analgesics are not given, please explain why. In addition, please provide the clinical signs of pain or distress which would indicate the animal needs analgesia. The Guide states: In general, unless the contrary is known or established, it should be assumed that procedures that cause pain in humans also cause pain in vertebrate species (IRAC 1985)* [*AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)*.*

§ *Criteria for termination include (but are not necessarily limited to) inability to ambulate, dehiscence or ulceration of surgical site, inability to eat or drink, loss of greater than 20% of body weight (over a period of time), piloerection, hunched posture.*

*\*\** *USP/NF (United States Pharmacopeia National Formulary) refers to the formulation of the substance. For example, various substances can be purchased as chemical grade (non-pharmaceutical) form sources such as Sigma/Aldrich/Fluka. USP/NF grade substances are provided in a formulation specifically made for injection.*

*\*\*\* The UCAC will review the use of the substance based on the following criteria: 1) non-availability of an acceptable human/veterinary pharmaceutical grade compound, and 2) scientific necessity.*

## Statement K: Anesthesia, Sedation, or Tranquilization

*(If this statement is blank, omit it)*

Attach a **separate** Statement K for each species

1. Species:
2. Drugs used for restraint, tranquilization, sedation:

Drug(s):

Dosage(s):

Route(s) of administration:

USP/NF?\*  No  Yes

1. Drugs used for anesthesia:

Pre-Anesthetic (i.e. sedative):

Drug(s):

Dosage(s):

Route(s) of administration:

USP/NF?\*  No  Yes

Induction:

Drug(s):

Dosage(s):

Route(s) of administration:

USP/NF?\*  No  Yes

Maintenance:

Drug(s):

Dosage(s):

Route(s) of administration:

USP/NF?\*  No  Yes

1. Expected duration of anesthesia:

< 30 minutes

30-60 minutes

1-2 hours

2-4 hours

> 4 hours

1. How will depth of anesthesia be monitored?
2. Who will perform the anesthesia?
3. Where will the animal be anesthetized? Building:       Room#
4. Paralytic agents:

Will a paralytic agent be used?  No  Yes

*IF YES*, complete the following:

Drug(s):

Dosage(s):

Route(s) of administration:

USP/NF?\*  No  Yes

1. What is the purpose of using a paralytic agent?
2. Describe the methods used to monitor the level of anesthesia in the paralyzed animal:

\* *USP/NF (United States Pharmacopeia National Formulary) refers to the formulation of the substance. For example, various substances can be purchased as chemical grade (non-pharmaceutical) form sources such as Sigma/Aldrich/Fluka. USP/NF grade substances are provided in a formulation specifically made for injection.*

## Statement L: Behavioral Screening/Conditioning

*(If this statement is blank, omit it)*

Attach a **separate** Statement L for each species

1. Species:
2. What is the purpose of the behavioral screening/conditioning?
3. Which of the following reinforcement techniques will be used?

No reinforcement required

Food reward (complete Statement D)

Liquid reward (complete Statement D)

Electrical shock:

Strength:

Duration:

Frequency:

1. Describe the experimental procedures used for behavioral screening/conditioning:
2. What criteria will be used to monitor the long-term condition of the animals during the training and experimental periods?

## Statement M: Antibody Production and Vaccine Challenge

*(If this statement is blank, omit it)*

Attach a **separate** Statement M for each species

1. Species:
2. Describe the immunization procedure (including preparation of the skin):
3. Antigen(s):
4. Adjuvant (initial injection):
5. Adjuvant (booster injections):
6. Route of administration:
7. Volume per injection site:
8. Total volume of adjuvant and antigen:
9. Frequency of administration:
10. Anesthesia/sedation:

Will the animals be anesthetized or sedated during the immunization procedure?

No Yes

*IF YES*, the attach a Statement K: Anesthesia, Sedation, Tranquilization of Animals

1. Fluid/tissue collected:

Blood (attach Statement F: Antemortem Fluid Collection)

Ascites fluid (Attach Statement F: Antemortem Fluid Collection)

Tissue (specify; if taken from anesthetized animals, complete Statement H and Statement K):

1. Will animals receive a vaccine challenge following immunization? No Yes

If yes, then complete Statement X: Administration of Exogenous Substances or Tissues

## Statement P: Clinically Adverse Rodent Phenotype

**(use of a genetically modified strain in which the alteration**

**is known to influence morbidity and/or mortality)**

*(If this statement is blank, omit it)*

Attach a **separate** Statement P for each species

1. Species:
2. Is the strain being made by your lab, or is it commercially available?
3. If the phenotype is known, please describe the clinically adverse effects. (*i.e. seizure, skin ulcer, kidney failure, inflammatory bowel disease, if it is age-affected, whether it is present in homozygotes versus heterozygotes, if it is sex-linked, and what the morbidity/mortality is, etc. A search at* [*Mouse Genome Informatics (MGI)*](http://www.informatics.jax.org/) *may be helpful*)
4. What percentage of the offspring is affected?
5. What special care, if any, will be provided for this strain?
6. Describe in detail the criteria§ (i.e. clinical signs) which will result in euthanasia of the animal to prevent undue pain or distress:

§ *Criteria include (but are not limited to) inability to ambulate, inability to eat or drink, loss of greater than 20% of body weight (over a period of time), piloerection, hunched posture.*

## Statement R: Animal Restraint

*(If this statement is blank, omit it)*

Attach a **separate** Statement R for each species

*\*note – this does not include momentary restraint for simple procedures, i.e. injections*

1. Species:
2. What is the maximum length of time any single animal would be restrained within a 24 hour period?
3. Method of restraint

Describe procedure(s) in detail, including duration and any restraint device or cage employed:

## Statement S: Biohazard and Special Requirements

*(If this statement is blank, omit it)*

Approval pending Approved (provide most recent approval letter)  Exempt

1. Does the project involve the use of any of the following? If so, please identify the agent in the appropriate category:

Infectious agents (ETSU Institutional BioSafety and Chemical Safety Committee)

Agent(s):

Hazardous chemicals, including chemical carcinogens (ETSU Institutional BioSafety and Chemical Safety Committee)

Chemical(s):

Recombinant DNA or genetically altered materials (ETSU Institutional BioSafety and Chemical Safety Committee)

Material(s):

Radioactive materials (Radiation Safety Committee)

Material(s):

1. Specify the containment methods to be followed in protecting other research animals and personnel from any of the agents listed above:

## Statement T: Tumor Studies

*(If this statement is blank, omit it)*

Attach a **separate** Statement T for each species

1. Species:
2. How will the tumors be produced?

Naturally-occurring tumor

Implanted tumor (*complete Statement X; additionally, for surgical implantation, complete* Statement I *and* Statement K)

Type:

Source\*(include species):

Number of cells or size of tissue administered/implanted:

Site of implantation or injection:

Have the cell line(s) been tested for mouse viral agents?

No Yes *IF YES*, by whom?       When?

Carcinogen induced

Agent(s):

(Complete Statement S and Statement X for the agent(s))

1. Tumor size:

Will the size of the tumor exceed 1.5 cm in any dimension or 10% of the body weight of the animal? No Yes *IF YES*, provide written scientific justification:

1. §Describe conditions that would require you to abort the experiment and euthanize the animal:

*\*Human and primate tumor tissue or cell lines are potentially infectious to humans. Please complete* Statement S *for these agents.*

*§Conditions include (but are not necessarily limited to) inability to ambulate, ulceration of site, inability to eat or drink, loss of greater than 20% of body weight (over a period of time), piloerection, hunched posture.*

## Statement X: Administration of Exogenous Substances or Tissues

*(If this statement is blank, omit it)*

Attach a **separate** Statement X for each species

1. Species:
2. Table of Exogenous Substances:

| Substance |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dose |  |  |  |  |
| Volume |  |  |  |  |
| Route |  |  |  |  |
| Frequency |  |  |  |  |
| USP/NF\* | No Yes | No Yes | No Yes | No Yes |

1. If using non-pharmaceutical grade substance(s), please elaborate on the scientific necessity, whether or not the compound is available in pharmaceutical grade, and the formulation of the final product (i.e. sterility, pH, pyrogenicity, osmolality, etc.). Be sure to describe how the compound will be prepared, including diluents used prior to administering to the animal. (*Refer to the UCAC Policy on the use of Non-Pharmaceutical Grade Chemicals or Compounds in Laboratory Animals*.)
2. Anesthesia/Sedation:

Will the animal be anesthetized or sedated during the procedure? No Yes *IF YES*, then attach a Statement K: Anesthesia, Sedation, Tranquilization.

1. Physical/Physiological Effect:

Will physical or physiological effect(s) (i.e. decreased blood pressure, increased heart rate, etc.) likely result from this treatment whether clinically apparent or not? No Yes *IF YES*, then describe in detail, including criteria§ for termination of the experiment:

*\***USP/NF (United States Pharmacopeia National Formulary) refers to the formulation of the substance. For example, various substances can be purchased as chemical grade (non-pharmaceutical) form sources such as Sigma/Aldrich/Fluka. USP/NF grade substances are provided in a formulation specifically made for injection. The UCAC will review the use of the substance based on the following criteria: 1) non-availability of an acceptable human/veterinary pharmaceutical grade compound, and 2) scientific necessity.*

*§Criteria include (but are not necessarily limited to) inability to ambulate, inability to eat or drink, loss of greater than 20% of body weight (over a period of time), piloerection, hunched posture.*

## Statement Y: Field/Wild Animal Studies

*(If this statement is blank, omit it)*

Attach a **separate** Statement Y for each species

1. Species:
2. Are animals to be:

Live-captured and released at capture locality

Live-captured and released at a different locality

Non-survival collection

1. Study localities: Provide brief description, county and state. Indicate capture and release localities, if different.
2. List state and/or national permits required AND indicate those already obtained (give permit number) or status of any permit applications pending. Copies of permit need not be submitted but should be available upon request.
3. Describe the procedure (description must include method of capture, frequency of monitoring if trapping devices will be used, whether live animals will be transported to ETSU, and method of euthanasia, if necessary. For live-capture studies, also include individual marking procedures and their potential mortality effects):

## Statement Z: Other Procedures

*(If this statement is blank, omit it)*

Attach a **separate** Statement Z for each species

1. Species:
2. Describe any procedures which are planned by which do not appear elsewhere in the protocol.