**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

**CC-IACUC PROTOCOL APPLICATION**

**(FOR USE OF LIVE VERTEBRATE ANIMALS) v 9/22**

**\*\* Submit via IACUC Moodle Site on Moodle Spaces \*\***

*For assistance with completing this form, contact Melissa Burns-Cusato or any CC-IACUC member. To advance from one field to another press the tab key.* ***To place a check in a box, double-click with your mouse to the left of the box****.* ***Please use a font of your choice but not Times Roman****, which is the font used in the form.*

Title of Project /Course

Principal Investigator/Instructor(s)

Work phone E-mail Program

Names of Co-PIs

Your signature as Principal Investigator (PI), co-PI, or Instructor on this form verifies that: 1) the information contained herein is accurate, 2) you will comply with the legal standards and be held responsible for animal care and use established under federal and state laws and university policies, 3) you are responsible for assuring all listed personnel in this protocol are appropriately trained in animal procedures and have read this protocol prior to beginning work, and 4) the animal procedures in the protocol match the submitted grant information; 5) you understand that if you do not respond within 90 days to CC-IACUC inquiries, this protocol will be considered withdrawn.

Signature of PI/Instructor Date

Signature of Co-PI/Instructor(s)  Date

FOR CC-IACUC USE ONLY

CC-IACUC Number: Review Process: Full DMR

Pain/Distress Category: Approval Date:

Biosafety:  Exception to Guide:

Chemical: Restraint:

Radiation: Wildlife**:** MMSS:

USDA: Ag Animal**:** Consent form:MOU:

Brief instructions: Please complete pages 1-9. On page 10, check the procedures used in your study and complete the appropriate appendix. You may delete those appendices which are not part of your study. Placing your cursor over the box and clicking twice will place the “X” inside the box.

I. General Information

A. This protocol is:

New 3-year rewrite – Please provide a brief description of research progress to date. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Research Teaching – Course name:**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Collaboration between Centre College and another institution. If working with another institution, please provide the context of the collaboration (i.e., funding, personnel animals)

**B. Funding source for this protocol: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

For **NIH proposals**, provide the Vertebrate Animals and Research Strategy sections of your grant must be attached. The reviewer will be comparing:

1) Species

2) Animal Numbers

3) Procedures

**C. Non-technical Summary**

Describe in lay terms (aim for a high school-senior reading level) the purpose of the proposed research or teaching activity. Spell out all acronyms at first occurrence**.**

**D. Flow Chart**

Provide a flow chart or bulleted list that depicts the sequence of all animal procedures or manipulations (step by step) to be performed in this protocol. Specifics on drug dosages and administration information are to be entered in the appropriate appendix.

**II. Animal Welfare**

1. **Benefit**

Describe the potential scientific benefit of the proposed study. Be convincing as to why this work is important for advancement of knowledge, improving human or animal health, or for the good of society.

1. **Rationale**

Please explain your rationale for using live animals and why the proposed species is/are the most appropriate for this study.

1. **Duplication**

The Animal Welfare Act and USDA Animal Care Policy #12 require PIs to assure the IACUC that you have considered whether or not your proposed work unnecessarily duplicates existing knowledge.

Does the proposed activity unnecessarily duplicate any previous work? (teaching activities with **new** students are **not** considered duplicative) Yes No

If yes, please justify.

**D. Search for** **Alternatives to Painful/Distressful Procedures**

**A painful procedure in an animal** is defined as **any procedure that would be reasonably expected to cause more than slight or momentary pain and/or distress in a human**. The CC-IACUC is responsible for ensuring that investigators have appropriately considered alternatives.

Does this study/activity include painful or distressful procedures?: Yes No

If yes, please list the painful procedures:

Use the chart below to identify the species and the level of maximum discomfort, distress, or pain involved in the procedure. Reference the USDA pain level categories below to rate the pain/discomfort. Examples and guidelines for the USDA pain categories are included in Appendix S.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Number | Pain/Discomfort  (USDA category) | Anesthetic/  Analgesic/  Tranquilizer | Dose | Survival  Surgery |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**USDA Pain Categorization:**

|  |  |  |  |
| --- | --- | --- | --- |
| **USDA Category B** | **USDA Category C** | **USDA Category D** | **USDA Category E** |
| Breeding or Holding Colony Protocols | No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions; positive reward projects; routine procedures; injections; and blood sampling. | Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress. | Pain or distress or potential pain or distress that is **not** relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress. |

Note: There is no USDA Pain Category A

*The US Interagency Research Animal Committee states that "...investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."*

If using pain categories C, D, or E justify the use of pain or discomfort in the procedure.

***According to the above definition, literature searches should be performed on specifically those proposed procedures that may cause more than slight or momentary pain and/or distress.***

If YES, USDA Animal Care Policy #12 requires PIs to assure the IACUC that alternatives to procedures that cause more than momentary or slight pain or distress have been considered.

The Animal Welfare Act regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress in a human being to which that procedure is applied. Alternatives or alternative methods are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use in pursuit of the minimization of animal pain and distress consistent with the goals of the research. To satisfy this requirement, **the USDA believes that database searches remain the most effective and efficient method for demonstrating compliance with the requirement to consider alternatives to more than momentary painful / distressful procedures.** A minimum of **2 databases** must be searched.

|  |  |  |
| --- | --- | --- |
|  | Database 1 | Database 2 |
| Name of Database searched \* |  |  |
| Date of search (must be within 6 months of protocol submission) MM/DD/YY |  |  |
| Years covered by search  YY – YY |  |  |
| Search strategy (must show how keywords were combined) |  |  |
| Other sources consulted. Provide individuals’ name, qualifications, date and summarize content |  | |

*\* For suggestions on performing appropriate database searches and for a list of acceptable databases visit:* http://www.nal.usda.gov/awic/alternatives

For each painful procedure, were alternatives identified (to replace, refine, reduce)?

Yes No

If “Yes”, please describe:

Will they be incorporated into your proposed work? Yes No If not, please explain?

If “No”, Please provide a written narrative about your search results including references.

**E. Monitoring Health and Well-being**

Animals should be monitored at a regular interval for health and well-being.  The frequency of this monitoring by personnel listed on the protocol is variable and based on the protocol.  Please indicate below the clinical signs being evaluated during the monitoring.

If yes, please check all that apply (Placing your cursor over the box and clicking twice will place the “X” inside the box):

loss of appetite. If checked, how will appetite be monitored? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

loss of weight- \* *note:* *best for dogs, cats and other large animals*

* maximum amount or % weight loss: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* frequency of evaluation(s): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Body condition score- \* note: *most appropriate for rodents*. Please review the body condition scoring system. http://web.research.colostate.edu/ACP/PDF\_Docs/AALAS%20body%20condition%20scoring.pdf

* (explain/attach scoring method): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* frequency of evaluation: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* score at which veterinary consultation will be initiated: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* score at which animal will be euthanized: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

restlessness/ distress

abnormal resting postures in which the animal appears to be sleeping or is hunched up

licking, biting, scratching, or shaking a particular area

failure to show normal patterns of inquisitiveness

failure to groom, causing an unkempt appearance

guarding (protecting the painful area)

loss of mobility

red stain around the eyes of rats

unresponsiveness

self-mutilation

labored breathing

other, please describe: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**F. Removal from study**

If necessary, what criteria will be *used to remove* an animal from use prior to the planned conclusion of the study? **For each criterion, define a specified duration or endpoint.** For example, “animals will be removed from the study if diarrhea is observed for greater than 24 hours”.

**III. Veterinary Care**

Which veterinarian(s) is/are responsible for clinical care?

CC-IACUC Veterinarian (Dr. Bernard Doerning)

Other, Name: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**IV. Animal Description, Housing and Use Location(s)**

**A. Description of Animals**

Protocols are approved for 3 years, therefore, the number of animals requested should be the total number of animals needed for the entire project across a 3 year period. For questions regarding animal numbers, contact the IACUC Chair at [m.cusato@centre.edu](mailto:m.cusato@centre.edu).

|  |  |  |
| --- | --- | --- |
| **Species** | **Source** | **Total # Animals Requested \*** |
|  |  |  |

**B. Provide an explanation of how animal numbers were derived and justify that need**. A table may help clarify different experimental groups or studies and the specific numbers needed for each.

The number of animals should be justified scientifically, statistically, and/or by consultation with subject matter expert. Include the name of the individual who was consulted with to determine the animal number animals requested.

**C. Animal Housing and Use Areas:**

Please check your housing location.

Young Hall Rodent Facilities (

Young Hall Avian Facilities (

Young Hall Aquatic Facilities

\*Other **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

***\*Any location housing animals for greater than 12 hours must be inspected. Information on establishing a satellite facility may be obtained from the CC-IACUC office.***

**Use areas where animals will be used (including euthanasia).**

|  |  |  |
| --- | --- | --- |
| **Building/Room Number** | **Animal Manipulations** | **Estimated Use Time at Site (specific to this study/protocol)** |
|  |  |  |

**D. Are there any special requirements for housing/husbandry?** Yes No

If yes, please complete:

Sterile Cages

Wire Bottom Cages\*

No Enrichment\*

Social Isolation\*

Individually housed\*

Other **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

***\*Please provide scientific justification for use of this type of housing/husbandry***

**E. Planned disposition of the animals after completion of the activity:**

**V. Personnel**

List all personnel involved in this protocol and provide training information. Include all investigators, student employees, and student researcher assistants.

|  |  |  |
| --- | --- | --- |
| **Name + email address** | **Animal Care & Laboratory Training**  **Completion Date** | **Procedures this person will perform & relevant experience** |
| Use tab key to insert more lines as needed |  |  |

**VI. Euthanasia**

This must be answered even in a non-terminal study, where an animal may experience a Humane Endpoint not related to the research. Methods of euthanasia must be listed as acceptable by the most recent Report of the AVMA Guidelines on Euthanasia (<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>). Methods listed as conditionally acceptable must be scientifically justified below.

**Euthanasia is part of the study design**

**OR**

**Euthanasia is NOT part of the study design**

Method of euthanasia:

Anesthetic Barbiturate Overdose (An approved veterinary product): 1 cc/10 lb. by intravenous route

Anesthetic overdose

Drug: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Dose: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Route: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Decapitation under anesthesia or tranquilization (if yes, complete **Appendix N**)

Cervical dislocation (CD) under anesthesia or tranquilization (if yes, complete **Appendix N**)

Carbon dioxide (CO2) exposure followed by secondary method  CD or  Thoracotomy

Exsanguination under anesthesia or CO2 (if yes, complete **Appendix N**)

**Is Perfusion involved?**

**No**

**Yes. Please provide details. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Other: Specify: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**VII. Animal Procedures**

Please check all that are appropriate. Placing your cursor over the box and clicking twice will place the “X” inside the box.

**A.** \*Antibody production. If yes, complete **Appendix A.**

B. \*Substance(s) collected from live animals (Blood\*, or other body fluid withdrawal, tissue collection, tail clip\*; do not list collections post mortem). If yes, complete Appendix B.

C. Substance(s) administered to live animals (Gavaging, injections). If yes, complete Appendix C.

**D.** Are animals restrained for purposes other than routine care and use (routine care and use includes cattle in a chute, mouse in restrainer for blood collection. If yes, complete **Appendix D.**

**E.** Projects involving food and water deprivation or dietary manipulation. If yes, complete **Appendix E.**

**F.** Temperature/light/other environmental manipulations. If yes, complete **Appendix F.**

**G.** Tumor models\*, disease models, or toxicity testing. If yes, complete **Appendix G.**

**H.** Behavioral studies. If yes, complete **Appendix H.**

**I.** Endoscopy, fluoroscopy, radiology, ultrasound, MRI, CT, PET, or other imaging procedures. If yes, complete **Appendix I.**

**J.** Use of tissues, serum, tumor lines, or hybridomas that are of rodent origin. If yes, complete **Appendix J.**

**K.** Creation of and/or use of genetically altered animals. If yes, complete **Appendix K.**

**L.** Wildlife field studies. If yes, complete **Appendix L.**

**M.** Controlled exercise (treadmills, forceplate, balance balls) If yes, complete **Appendix M.**

**N.** Anesthesia/analgesia\*. If yes, complete **Appendix N.**

**O.** Surgical Procedures. If yes, complete **Appendix O.**

**P. P**rojects involving aquatic animals. If yes, complete **Appendix P.**

**Q.** Other manipulations not described above. If yes, complete **Appendix Q.**

**R.** Hazardous agent use (biological, radioactive, or chemical). If yes, complete the **Animal Hazard Control Form – Appendix R**

## Appendix A – Antibody Production

1. Polyclonal antibody production

1. Antigen/Adjuvants to be used: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
2. If Freund’s Complete Adjuvant is to be used, concentration of mycobacterium**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Route of injection: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Location of injection(s): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Volume of injection per site: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Total number of injection sites: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

f. Frequency and maximum number of boosts: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

g. Interval between injection and booster: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

h. Frequency of monitoring injection sites/who will monitor: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

i. What will be done to minimize pain / distress: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

j. Adverse effects/endpoints: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**2. Monoclonal antibody production**

*Please provide scientific justification in the Animal Welfare Section of this protocol explaining why in vitro monoclonal antibody production methods cannot be used.*

a. Describe methodology: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Adjuvant used:  Yes  No

If yes, name of adjuvant: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Volume of adjuvant: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Number of cells to be injected into peritoneal cavity: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Volume of cells: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Criteria/signs that will dictate ascites harvest: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Will animal be anesthetized before harvest:  Yes  No

f. Total number of taps: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

g. How will animals be monitored/cared for following taps: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

h. What will be done to minimize pain / distress: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

i. Person responsible for monitoring/harvesting ascites: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix B - Substance(s) Collected (Blood\*, or other body fluid withdrawal, tissue collection, tail clip\*)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Substance Collected\*** | **Site of collection** | **Method of collection** | **Amount Collected** | **Frequency of Collection(s)** |
| Use tab key to insert more lines as needed |  |  |  |  |

***\*Please refer to the CC-IACUC policies, which include information on blood volume and tail biopsies. Deviations from these guidelines must be justified. Please see the following website for more details:*** <https://centrenet.centre.edu/ICS/Academic/Academic_Affairs/Institutional_Animal_Care.jnz>

Appendix C -Substance(s) Administered (Gavaging, injections) – If administered for anesthesia/immobilization please complete Appendix N

|  |  |  |  |
| --- | --- | --- | --- |
| Substance  Administered | Route and volume administered | Dose/Concentration | Frequency |
| Use tab key to insert more lines as needed |  |  |  |

***\*Investigators are expected to use pharmaceutical-grade drugs and compounds whenever available, even in terminal procedures. If non-pharmaceutical grade drugs/compounds will be used in live animal, list the drugs and/or compounds, how they will be prepared, how they will be used and explain why pharmaceutical grade drugs cannot be used.*** [ text box here ]

a. Are any adverse events expected following administration of the above substances? (hours for short term/weeks or months for long term): [Please list the potential adverse effects **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Frequency of observations: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Identify the laboratory personnel responsible for monitoring the animal(s) following administration of the above substances. List individual’s names, email address and provide cell phone numbers.

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix D – Restraint with Mechanical Devices** (for procedures other than routine care.)

a. Restraint device: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. For what procedures: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Duration of restraint: (If greater than 4 hours, justification must be provided) **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Frequency of observations during restraint/person responsible: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Frequency and total number of restraints: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

f. Conditioning procedures: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

g. Steps to assure comfort and well-being: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

## Appendix E - Projects involving food and water deprivation and dietary manipulation (Excluding routine pre-surgical fasting)

**1. Food Restriction**

a. Amount restricted: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Duration (hours for short term/weeks or months for long term): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Frequency of observations: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Person responsible: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**2. Fluid Restriction**

a. Amount restricted: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Duration (hours for short term/weeks or months for long term): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Frequency of observations: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Person responsible: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**3. Dietary Manipulations**

a. Compound supplemented and amount: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Compound deleted and amount: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Duration (hours for short term/weeks or months for long term): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Frequency of observations: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Person responsible: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

f. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

## Appendix F - Temperature/light/other environmental manipulations

a. Describe manipulation(s): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Duration: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Intensity: ­**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Frequency: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Appendix G - Tumor models\*, disease models, or toxicity testing

a. Describe methodology: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Expected model and/or clinical/pathological manifestations: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Frequency of observations: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Person responsible for oversight of animals: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

## Appendix H - Behavioral studies

a. Describe animal methodology/test(s) to be used: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Intensity and duration of stimulus: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Frequency of tests: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Length of time in test apparatus: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix I - Endoscopy, fluoroscopy, radiology, ultrasound, MRI, CT, PET, or other imaging procedures**

a. Describe which imaging procedure will be used on the animal: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Duration of procedure: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Frequency of observations during procedure: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Frequency/total number of procedures: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix J - Use of tissues, serum, tumor lines, or hybridomas that are of rodent origin**

These tissues must be free of rodent infectious agents. Please provide evidence.

a. Source: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Species: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Tissue type: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Provide evidence of appropriate testing or certification (MAP, PCR):

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix K – Genetic modification of animals**

a. Describe approach or procedure to produce modification: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. What gene(s) were intentionally modified: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. If known, expected product over expressed / under expressed: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Describe any potentially debilitating phenotypes associated with this animal:

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

### Appendix L – Wildlife Field Studies

**1. List any permits required** (e.g. Kentucky Wildlife Resources Agency, U.S. Fish and Wildlife Service, Endangered Species)

Name and number: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**2. Study Site(s)**

General Location: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**3. Describe methods employed to prevent potential disease transmission between individual animals encountered in this activity** (Use of disposable gloves; disinfect gloves between animals): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**4.** **Capture with mechanical devices**

a. Type/description of capture device/method: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Frequency of checking capture device: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Maximum time animal will be in capture device: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Methods to ensure well-being of animals in capture device: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Methods to avoid non-target species capture: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

f. Expected injury and/or mortality rates: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

g. Animal will be released at capture site after completion of procedures:  Yes  No If no explain:

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

h. Precautions used to minimize injury and/or mortality? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

i. Describe method(s) used to sanitize capture devices between capture locations or groups of individuals. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**5. Capture with chemical immobilization** (Anesthetizing drugs are covered under Appendix N, this section covers the mechanics).

a. Type of dart or device to administer drugs: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Method of dart propulsion: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Precautions used to minimize injury and/or mortality **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. If wild animals will be anesthetized and released to the wild, describe procedures for assuring that animals are sufficiently recovered from anesthetic to be released. Consider that prey species may have to be monitored until fully recovered to avoid predation. (Also, complete **Appendix N**)

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**6. Marking/Telemetry Procedures**

a. Describe marking procedures to be used: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. If a telemetry package is to be attached, describe:

1. Weight of the total package **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

2. Type of antenna (including length) **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

3. Method of attachment (for surgical attachment, complete appendix N and O)

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

4. Will marking/telemetry device be removed  Yes  No

If yes, explain how: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**7. Release of animals other than at capture site** (for non-survival collection please see #7)

a. Where will captured animals be released **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. If the animals are transported indicate the method of transportation.

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. If the animals are to be housed, fill out section IV. Animal Housing and Use Location(s)

d. If release is not the general location of capture, justify. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**8. Non-Survival Collection**

a. Describe procedure(s) to be used. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Describe precautions that will be taken to prevent non-target mortalities.

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix M –** Controlled exercise (treadmills, forceplate, balance balls). ***The USDA considers forced exercise to potentially be painful and/or distressful. Please review the information at this link: http://awic.nal.usda.gov/animal-welfare-act-quick-reference-guides#Q10***

1. Describe apparatus used and how used: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
2. Describe any stimulus (if used): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
3. Conditioning to apparatus: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
4. Frequency of events per day: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
5. Total number of sessions during this study: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
6. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix N - Anesthesia/Sedation/Analgesia/Other supplementary Drugs or compounds**

***Adequate records describing anesthetic monitoring and recovery must be maintained and available in the animal’s medical record. Animals must be monitored until awake and can maintain sternal recumbancy:***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Agent** | **Concentration/ Dose (mg/kg)** | **Volume and route** | **Frequency** |
| Pre-emptive analgesic |  |  |  |  |
| Pre-anesthetic/sedation |  |  |  |  |
| Anesthetic |  |  |  |  |
| Post-procedural analgesics\* |  |  |  |  |
| Paralytics (need to justify below in question h.) |  |  |  |  |
| Other (supplementary drugs or compounds ie. Atropine, Vetastarch) |  |  |  |  |

***\*Investigators are expected to use pharmaceutical-grade drugs and compounds whenever available, even in terminal procedures. If non-pharmaceutical grade drugs/compounds will be used in live, list the drugs and/or compounds, how they will be prepared, how they will be used and explain why pharmaceutical grade drugs cannot be used.* \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

* 1. Reason for administering agent(s): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
  2. For which procedures: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
  3. Method of monitoring anesthetic depth, including paralyzing drugs:

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Methods of physiologic support during anesthesia and recovery:

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Frequency of recovery monitoring: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

f. Specifically what will be monitored: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

g. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

h. If using paralytic drugs, scientific justification needed: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

i. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix O- Surgical Procedures**

* ***All survival surgical procedures must be done aseptically, regardless of species or location of surgery.***
* ***Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained and available for the attending veterinarian and animal care staff.***

**1. Location (Room, Building) of surgery:** **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

2. Type of Surgery

Non-survival surgery: (animals euthanized without regaining consciousness).

Major survival surgery: (major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function).

Minor survival surgery.

Multiple major survival surgery?

If yes, provide justification for multiple major survival surgical procedures: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**3. Pre-op preparation of the animals:** **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

a. Food restricted for **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**hours

b. Water restricted for **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**hours

4. Sterile techniques will include (check all that apply):

Sterile instruments

Sterile gloves

Mask

Cap

Sterile gown

Approved operating area

Removal of hair or feathers

Skin preparation with a sterilant such as betadine

Practices to maintain sterility of instruments during surgery

5. Describe the following surgical procedures:

a. Skin incision size and location on the animal: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Describe surgery in detail (include size of implant if applicable):

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Method of skin closure (include number of layers, type of wound closure, proposed suture type, size ranges): **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

i. Type: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

ii. Suture size / wound clips: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

iii. Pattern: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

6. Recovery from Surgical Manipulations

a. Following recovery, what parameters will be monitored? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Person who will monitor the animals? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. How frequently will animals be monitored? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. How long post-operatively will animals be monitored: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

e. Time post-operatively that sutures/staples will be removed, if applicable. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix P- Aquatics**

**1. Water quality monitoring**

a. How is the water quality established/determined prior to introduction of animals?

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. How is the water filtered to remove nitrogenous/animal waste compounds? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. How frequently and what parameters for the water quality are monitored? **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**2.  Housing**

a. Briefly describe the system design and housing used (include type of water circulation, tank size).

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Provide the approximate housing density. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Provide how often and how the housing will be sanitized.  **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. What type of environmental enrichment is being provided in the tank/housing? (PVC pipe, plants).  If none is used, please justify. **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Appendix Q- Other Procedures**

***Please describe in detail what will be done to animals (this response should provide the CC-IACUC with a clear understanding of procedure(s) to be performed on an animal or group of animals not covered in other sections.***

a. Describe animal methodology/manipulation/test(s) to be performed: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

b. Duration: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

c. Frequency of tests: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

d. Potential adverse events: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Appendix R- AHCF Animal Hazard Control Form-IACUC # | | | | | |
| The Principal Investigator is responsible for informing all personnel handling animals of the hazards involved and precautions to be taken. The Principal Investigator is also responsible for updating the form in the case of changes in agent or experimental procedure. For assistance in completion of this form please contact the appropriate Safety Officer. | | | | | |
| **Principal Investigator:**  **Program:** | **Campus Phone:** | **Emergency Phone:** | | | **E-Mail:** |
| **Secondary Contacts:**  **Program:** | **Campus Phone:** | **Emergency Phone:** | | | **E-Mail:** |
| **Chemical Agents:** | **Radiological Agents:**  **Radiological Materials License Holder:** | | | | |
| **Biohazardous Agents:** | **Infectious to humans (Y/N):** | | | **Biosafety Level:** | |
| **Required Personal Protective Equipment (PPE):** | | | | | |
| **Route of Excretion:** | | | | | |
| **Precautions for Handling Live or Dead Animals:** | | | | | |
| **Animal Disposal:** | | | | | |
| **Bedding / Waste Disposal:** | | | | | |
| **Cage Decontamination:** | | | | | |
| **Additional Precautions to Protect Personnel, Adjacent Research, and Environment:** | | | | | |
| **Study Location(s):** | | | **Form reviewed/approved by:**  **Date reviewed/approved:** | | |

**Appendix S: USDA Pain Level Examples and Guidelines**

|  |  |  |  |
| --- | --- | --- | --- |
| **USDA Category B** | **USDA Category C** | **USDA Category D** | **USDA Category E** |
| Breeding or Holding Colony Protocols | No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions; positive reward projects; routine procedures; injections; and blood sampling. | Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress. | Pain or distress or potential pain or distress that is **not** relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress. |
|  | **Examples** | **Examples** | **Examples** |
|  | 1. Holding or weighing animals in teaching or research activities. 2. Injections, blood col­lection or catheter implantation via superficial vessels. 3. Tattooing animals. 4. Ear punching of rodents. 5. Routine physical examinations. 6. Observation of animal behavior. 7. Feeding studies, which do not result in clinical health problems. 8. AVMA approved humane euthanasia procedures. 9. Routine agricultural husbandry procedures. 10. Live trapping. 11. Positive reward projects. | 1. Diagnostic procedures such as laparoscopy or needle biopsies. 2. Non-survival surgical procedures. 3. Survival surgical procedures. 4. Post operative pain or distress. 5. Ocular blood collection in mice. 6. Terminal cardiac blood collection. 7. Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with decreased appetite/ activity level, adverse reactions, to touch, open skin lesions, abscesses, lameness, conjunctivitis, corneal edema and photophobia. 8. Exposure of blood vessels for catheter implantation. 9. Exsanguination under anesthesia. 10. Induced infections or antibody production with appropriate anesthesia and post-op/post-procedure analgesia when necessary. | 1. Toxicological or micro­biological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs. 2. Ocular or skin irritancy testing. 3. Food or water deprivation beyond that necessary for ordinary pre-surgical preparation. 4. Application of noxious stimuli such as electrical shock if the animal cannot avoid/escape the stimuli and/or it is severe enough to cause injury or more than momentary pain or distress. 5. Infliction of burns or trauma. 6. Prolonged restraint. 7. Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes. 8. Use of paralyzing or immobilizing drugs for restraint. 9. Exposure to abnormal or extreme environmental conditions. 10. Psychotic-like behavior suggesting a painful or distressful status. 11. Euthanasia by proce­dures not approved by the AVMA. |

**Guidelines** for determining USDA classification in protocols involving tissue collection before/after euthanasia and/or animal perfusion:

If an animal will be euthanatized by an approved physical or chemical method of euthanasia solely for the collection of tissues (after the animal's death), the procedure should be classified as USDA C.

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy), and the animal will then be allowed to recover, the procedure should be classified as USDA D (survival surgery).

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy, etc.); and the animal will then be euthanatized, the procedure should be classified as USDA D (non-survival surgery). In this scenario, it is necessary to justify why the animal couldn't be euthanatized (USDA category C) rather than anesthetized.

If an animal will be anesthetized so that vital tissues can be collected (heart, both kidneys or lungs, whole liver, etc.), the animal will obviously succumb to the procedure. To determine whether this will be euthanasia or non-survival surgery, we must consider the definition of euthanasia. A critical component of this definition is "rapid unconsciousness followed by loss of cardiac, respiratory and brain function". Based on this definition, procedures which require tissue manipulation or other prolonged techniques prior to the animals death (more than a few minutes) should be classified as non-survival surgery (USDA D). Similarly, if an animal will be anesthetized so that the tissue can be collected in the "freshest" possible state (i.e. heart) and the tissues will be rapidly excised, the procedure should be classified as euthanasia (USDA C). (Note: In this scenario, it is difficult to justify why the animal couldn't be euthanatized rather than anesthetized.)

If an animal will be anesthetized so that it can be chemically perfused, the same "test of time" applies (i.e.: long, technical manipulations should be classified as USDA D; while rapid intravascular injection of the perfusate without other manipulations should be classified as USDA C).

**NOTE:** Because the USDA classification system is based on the "potential for pain, distress or discomfort," the anesthetic/euthanasia drug dose becomes a critical concern. For example, if a known "euthanasia dose" of pentobarbital will be administered, drug irreversibility is assumed. Thus, once the animal is confirmed to be in an anesthetic plane (toe pinch response, etc.), tissues can be collected/ procedures can be performed without the concern about what the animal will be perceiving. This procedure would then be classified as USDAC. The Committee recommends using a euthanizing dose whenever possible. Other methods may be appropriate with proper scientific justification.