IACUC Protocol Number	Submission Date	Final Approval Date

## **Administrative Information**

Please be aware that there are potential civil and criminal penalties involved if someone gives <u>false information</u> to obtain monetary support from for example the National Institutes of Health. I hereby affirm that the information I have provided in this document is accurate and true to the best of my knowledge and belief. Please check yes, to provide confirmation.

[] Yes [] No, Explain, <**Text Box>** 

- 2. Personnel and Personnel Training Information
  - a. According to Federal Regulations, all personnel must be qualified to perform those animal activities described in this protocol prior to working independently. By checking "Yes" to this statement you provide written assurance and documentation that all personnel listed on this protocol will complete all the required training and be under the close supervision of and trained by qualified and experienced individuals before they are permitted to conduct any of the animal activities listed on this protocol independently. [] Yes [] No, Explain, <Text Box>
  - b. List those individuals that will be conducting animal activities under this protocol.

#### Note: Assigned by the administrator or an IT system

Name (Last, First)	Role (e.g., Admin contact, lab manager, Post doc, research assistant, student):	Unique identifier (i.e., email, employee number)

- 3. Project Funding Source(s)
  - a. Is this project or components of it externally (e.g., NIH or DoD) funded? [] Yes [] No
  - b. If the project is externally funded by, please check all that apply.
    - □ National Institutes of Health (NIH)
    - □ National Science Foundation (NSF)
    - □ Department of Defense (DoD)
    - $\Box$  Other, please list.
  - c. Funding Agencies require all animal activities listed in the grant to be reviewed and approved by the IACUC prior to spending any of the associated funded on animal activities. Please check yes to provide assurance that proceeds from the grant(s) intended to support animal activities will only be spent after those activities are approved by an IACUC. [] Yes [] No, Explain
- 4. Please check the box(es) that best describes your proposed activities.
  - □ Breeding Colony
  - $\hfill\square$  Euthanasia only for a source of tissue
  - □ Instruction (e.g., teaching, training, demonstration)
  - □ Research
  - □ Specialized Services (e.g., Diagnostic, Imaging, and Transgenic Cores)
  - □ Wildlife/Field Research
  - $\Box$  Other, please describe.
- 5. Animal Use Locations
  - **a.** Please check "Yes" to provide assurance that all animal activities (i.e., housing and procedures) will occur in the institution's central animal facilities (e.g., areas managed by the Attending Veterinarian rather than the PI).
    - [] Yes[] No, Explain

b. Please provide the following information about locations where animal activities will occur outside the central facilities and the activities that will be performed.

Building, Room Number	Species	Activities to be performed (e.g., behavior studies, euthanasia, housing, non-survival surgery, survival surgery, substance administration, use of hazardous materials, specialized equipment, other)

## **IACUC Information**

1. Please provide the following information for the animals that will be used in the activities proposed under this protocol (complete for each species)

Species	Source (e.g., approved vendor, wild caught, another IACUC approved protocol):

2. Please describe the rationale for involving animals in your research design. Describe specific language that describes how the animal model selected was chosen because, for example, its physiology is the most appropriate to achieve the project goals. For example, a swine model may be selected since its physiology is the closest to that of a human and it would provide the best transitional information. In addition, that the model selected is the lowest on the phylogenetic scale that can be used to satisfy the project goals (e.g., rabbits were not selected if the activities can be successfully conducted in mice since mice are less sentient). **Text Box** 

3. Euthanasia and/or Final Disposition of the animals to be used (*Check all that apply*)

Note: The American Veterinary Medical Association (AVMA) Guidelines define the humane methods of euthanasia that may be used to euthanize animals. Federal regulations (e.g., the PHS Policy) require that the AVMA Panel recommendations be followed and any deviation from the guidelines require IACUC review and approval. Please check yes to provide assurance that all methods of euthanasia listed in this protocol are in accordance with the current American Veterinary Medical Association Panel on Euthanasia recommendations. *[] Yes [] No, Explain* 

□ Qualified animals may, for example, be transferred to another IACUC approved protocol or adopted (if eligible) following institutional policies.

- □ Wild animals being used will be released back to the wild
- □ Some animals will be involved in a terminal procedure that will be later described.
- □ For adult mice or rats, they will be euthanized by gas (i.e., carbon dioxide or isoflurane) inhalation, or by exsanguinations under anesthesia followed by one of the listed secondary physical methods (i.e., decapitation, bilateral pneumothorax, removal of a vital organ, cervical dislocation) of euthanasia.
- □ A physical method (e.g., cervical dislocation or decapitation) will be used.

Method	Species

□ Lethal Injection with or without tissue collection

Agent to be used	Dose	Species

□ Other (for wildlife studies, include the type of euthanasia that will be used should animals be unexpectedly injured during a procedure)

Method	Species

- 4. Agent Administration
  - a. Other than anesthetics and analgesics, will substances (including but not limited to, for example, biologics, sedatives, tranquilizers, neuromuscular blocking agents (may not be used without anesthesia), antibiotics, supplemental fluids, experimental compounds, or test articles) be administered, either directly or as a food/water additive, to animals? [] Yes <complete the table> [] No

Agent:					
	Purpose for Administration	::			
	Route of administration (e.g., orally, dermal, IV injection)		Dosage (units and Volume)	Frequency	

b. Substances (e.g., infectious agents, clinical specimens, and toxic chemicals) that are potentially hazardous to humans are regulated through, for example, the Institutional Biosafety Committee (IBC), and/or the institution's Office of Environmental Health and Safety (EHS). Please check yes, to attest that your use of the hazards listed in the table above has been cleared by the either the IBC, EHS or equivalent. [] Yes, [] No, explain.

Substance	Reason for lack of safety clearance

c. Federal regulations state that substances (e.g., anesthetics, analgesia, chemicals, biologics, and other substances) administered to animals as part of a research, teaching, or testing survival or non-survival activity must be pharmaceutical grade when available. The FDA provides a list\* of currently available pharmaceutical agents. Please check yes to provide assurance that, when available, all substances administered to animals under this protocol will be pharmaceutical grade. [] Yes [] No, complete the table below. \*(http://www.accessdata.fda.gov/scripts/cder/drugsatfda)

Substance(s)	Is the reagent available in pharmaceutical grade? <i>(Yes/No)</i>	If "Yes," why must the non- pharmaceutical grade be used?	If "Yes," say 'provide assurance that purity sterility, and pH of the solution will be physiologically appropriate <i>(Yes/No)</i>

#### **Objectives and Rationale (IACUC Section)**

- 5. Related to the use of vertebrate animals, briefly describe the objective(s) and a general description of this project in language that can be easily understood by a nonscientific member of the IACUC.
- 6. Please check "yes" to provide assurance that the scope of the proposed animal activities will not unnecessarily duplicate previously conducted activities, and either be used to identify potential medical advancements that improve the health and wellbeing of humans and/or animals, used as a resource in educational endeavors, or used to advancement human knowledge for the good of society. *[] Yes [] No, Explain*

7. Please check "Yes" to provide assurance that as the PI you have considered the feasibly of using non-animal models (e.g., cell culture, isolated organ preparations, computer simulations, etc.) to achieve your research goals. In addition, your acknowledgment confirms that using living animals is essential to evaluate the hypothesis and determine the overall impact of the research on a complete biological system (i.e., animals).

[] Yes [] No, Explain

- 8. Experimental Design
  - a. Please provide a clear and concise, sequential description of the procedures involving the use of animals in a manner that is easily understood by a nonscientific member of the IACUC. This description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment through the endpoint of the study. *Note: Please do not provide the specific details (e.g., drug doses, routes of injection, and steps in a surgical procedure) of the animal procedures you will conduct since those details will be provided as part of subsequent questions.*
  - b. The number of animals used for each experiment should be the minimum required to achieve the scientific aim(s) and produce, <u>when possible</u>, statistically valid results. Please check "Yes" to provide assurance that the number of animals used in this experiment is the minimum needed to produce valid results. [] Yes[] No, Explain
  - c. Estimate the minimum and maximum number of animals that will be needed for this project to achieve valid results.

Species	Minimal needed to achieve valid results	Maximum Need

#### 9. Animal Housing

a. The living conditions that are appropriate for the species and contribute to the welfare and comfort of that species have been standardized in the Federal regulations. These conditions include, for example, maintaining appropriate light cycles and environmental

conditions; providing ongoing access to enrichment devices; changing cages on a regular basis; and using standard size cages. Please check yes to confirm that animals under this study should be maintained according to the federally defined standards. [] Yes [] No, describe and justify the needed changes.

b. Federal regulations require animals to be housed in compatible groups, which contributes to the welfare and comfort of the animals. Please check yes to confirm that animals under this study can be maintained in compatible groups according to the federally defined standards. (Note: single housing of animals due to fighting, veterinary treatment or post-operative recovery, attrition, or separation of breeders does not need to be identified here). [] Yes [] No, describe and justify why animals must be singly housed.

10. Animal Procedures (Check all that apply)

NOTE: <u>According to the Federal Regulations</u>, it is your responsibility to minimize the discomfort, distress, and pain that may be associated with any animal use activities proposed in this protocol. Please check yes to provide assurance that measures, not limited to anesthetics, analgesics, and sedatives, will be taken to avoid or minimize the discomfort, distress, and pain to the animals. *[] Yes [] No, please explain.* 

□ Survival surgery (i.e., the animal will recover) will be performed.

- 1. Please provide a descriptive name (e.g., laparotomy or abdominal) of the specific survival surgical procedure that will be performed.
- 2. Briefly describe the surgery that will be conducted. *Note: details regarding anesthesia, analgesia, surgical preparation, and post operative monitoring will be provided in subsequent questions.*
- 3. Survival surgery must be performed using aseptic technique. Aseptic technique is a series of practices and procedures that help to prevent contamination from pathogens. Aseptic technique is often influenced by the species of interest and include, for example, patient preparation (pre-emptive actions: fur removal, surface cleaning, etc.), surgeon preparation (protective clothing, PPE, skin disinfection, cover) and surgical location (room, table, surface disinfection). Consequently, please check yes to provide

assurance that the following process will be followed to ensure aseptic technique is used.

- The instruments and/or medical devices will be sterilized (e.g., autoclaved) prior to each surgery. The surgical area will be decontaminated using appropriate disinfecting practices. The surgeon will wear, at minimum, a mask, a surgical cap/bonnet, sterile gloves, and a clean scrub top, disposable gown, or lab coat.
- **Mammals**, the surgical site will be prepped by removing the hair followed by at least three alternating scrubs with a disinfectant (e.g., betadine, chlorhexidine) and rinse (e.g., ethanol, warmed saline, sterile water) to remove remaining visible debris.
- **Birds**, the feathers located within the surgical site will be removed followed by at least three alternating scrubs with a disinfectant (e.g., chlorhexidine) and rinse (e.g., ethanol, warmed saline, sterile water) to remove remaining visible debris.
- For amphibians, reptiles and fish, the incision site will be prepared with a single wipe using, for example, sterile saline, dilute povidone iodine, or chlorhexidine immediately before surgery.

Please check yes to provide assurance the described aseptic procedures will be followed consistent with the species of interest. [] Yes [] No, explain.

- 4. Patient support before, during and after surgery is critical to animal welfare and necessary to minimize discomfort, pain and/or distress associated with the surgical procedure. Please provide the following information.
  - a. Anesthetics and Analgesics must be used to manage and control pain during and after surgical procedures. Please check yes to provide assurance that both will be used as part of this procedure. [] Yes [] No, please explain.
  - b. List the anesthetic(s) and analgesic(s) that will be used for this procedure through the completion of post-operative care.

Substance	Species	Anesthetic or Analgesic	Dose	Application: induction, maintenance, or post- operative	Frequency of application (e.g., every 12 hrs.)

- 5. Each animal must be monitored until it completely recovers from the surgery, please check yes to provide assurance that the following post operative monitoring procedure will be occur.
  - Mammals (rodent): Post operatively each animal will be visibly observed at least every 15 minutes until the animal can hold itself upright. Each animal may be individually housed until it completely recovers from the surgery. Each animal will be monitored daily during the post-op recovery period (i.e., a minimum of 7 days), or until the sutures, staples or clips are removed.
     [] Yes [] No, please explain.
  - Mammals (non-rodent): Post operatively each animal will be visibly observed continuously until the endotracheal tube is removed (if applicable), and then every 15 minutes until the animal can hold itself upright. Each animal will be monitored daily during the recovery period (7-14 days) and/or until the sutures, staples, or clips are removed. [] Yes [] No, please explain.
  - **Birds:** Post operatively each bird will be visibly observed at least every 15 minutes until it can hold itself upright. Each may be individually housed until it completely recovers from the surgery. Each will be monitored daily during the post-op recovery period (i.e., a minimum of 7 days), or until the sutures, staples or clips are removed. [] Yes [] No, please explain.
  - Amphibians and reptiles: Animals will be visibly observed every 15 minutes during recovery from anesthesia until they resume normal swimming and ambulation. Animals will be monitored at least once per day during the post-op recovery period, which is typically 10 to 14 days. If applicable, the sutures will be removed within 14 days post-surgery. [] Yes [] No, please explain.
  - Fish: they will be visibly observed every 15 minutes during recovery from anesthesia until they swim normally. Each fish will be monitored for 24 hours post-surgery and then daily health checks performed. If applicable, the sutures will be removed within 14 days post-surgery.
     [] Yes
     [] No, please explain.

□ Non-survival surgery (i.e., the animal will not recover) will be performed.

- 1. How long will it take to complete the procedure?
- 2. Please provide a descriptive name (e.g., laparotomy or abdominal) of the specific surgical procedure that will be performed.
- 3. Briefly describe the surgery that will be conducted. *Note: details regarding anesthesia, analgesia, surgical preparation, and post operative monitoring will be provided in subsequent questions.*
- 4. Performing non-survival surgery using aseptic technique is a recommended practice. Aseptic technique is a series of practices and procedures that help to prevent contamination from pathogens. Aseptic technique is often influenced by the species of interest and include, for example, patient preparation (pre-emptive actions: fur removal, surface cleaning, etc.), surgeon preparation (protective clothing, PPE, skin disinfection, cover) and surgical location (room, table, surface disinfection).
  - The instruments and/or medical devices will be sterilized (e.g., autoclaved) prior to each surgery. The surgical area will be decontaminated using appropriate disinfecting practices. The surgeon will wear, at minimum, a mask, a surgical cap/bonnet, sterile gloves, and a clean scrub top, disposable gown, or lab coat.
  - **Mammals**, the surgical site will be prepped by removing the hair followed by at least three alternating scrubs with a disinfectant (e.g., betadine, chlorhexidine) and rinse (e.g., ethanol, warmed saline, sterile water) to remove remaining visible debris.
  - **Birds**, the feathers located within the surgical site will be removed followed by at least three alternating scrubs with a disinfectant (e.g., chlorhexidine) and rinse (e.g., ethanol, warmed saline, sterile water) to remove remaining visible debris.

• For amphibians, reptiles and fish, the incision site will be prepared with a single wipe using, for example, sterile saline, dilute povidone iodine, or chlorhexidine immediately before surgery.

Please check yes to provide assurance the described aseptic procedures will be followed consistent with the species of interest. [] Yes [] No, explain.

- 5. Patient support before, during and after surgery is critical to animal welfare and necessary to minimize discomfort, pain and/or distress associated with the surgical procedure. Please respond to the following information.
  - a. Anesthetics and Analgesics must be used to manage and control pain during the surgical procedure. Please check yes to provide assurance that both will be used as part of this procedure. [] Yes [] No, please explain.
  - b. List the anesthetic(s) and analgesic(s) that will be used for this procedure through the completion of post-operative care.

Substance	Species	Anesthetic or Analgesic	Dose	Application: pre-emptive, or during surgery

- □ Multiple survival surgeries will be performed on the same animal and on different days under separate anesthesia episodes.
  - 1. Please list the survival surgeries each animal will experience in the table below.

Species	Surgery #1	Surgery #2	Surgery #3
	(Descriptive Name	(Descriptive Name	(Descriptive Name
	Previously Provided in	Previously Provided in	Previously Provided in
	the Description)	the Description)	the Description)

2. Multiple surgeries conducted on the same animal are acceptable only if they are essential to the research project and scientifically justified. Provide scientific justification for performing multiple surgeries on a single animal.

## □ Food Manipulation in the form of restriction or regulation (food manipulation for clinical reasons such as pre-surgical fasting need not be included in the protocol)

1. Define the proposed food manipulation in the table below:

Species	Length of time (Hrs.) it will occur in a single occurrence	Minimum period between manipulations	Explain why the manipulation is necessary to meet the research goals

- Please check yes to provide assurance that animals will be closely monitored to ensure the food manipulation will not negatively impact animal welfare. Consequently, body weights will be recorded at least weekly and/or more often for animals under extreme restrictions. [] Yes [] No, please explain.
- 3. Food manipulations may negatively impact animal welfare. Consequently, the consideration of potential adverse consequences, and the identification of criteria for the removal (i.e., the humane endpoints) of an animal from the study is necessary. Removal from the study could either be euthanasia, the return to normal feeding, or another appropriate intervention. Do you expect the food restriction to negatively impact animal welfare? [] Yes, complete the table below [] No

Species	Criteria that will be used to remove an animal from the study	Please indicate, yes, to confirm that the euthanasia, final disposition section of this protocol will be applied when removing an animal from this study. (If no, explain)

4. Food deprivation for periods exceeding 24 hours can result in prolonged distress for the animals. Will any animals be deprived of food for more than 24 hours?
[] Yes, complete the table below [] No

Species	How many animals will be food deprived for more than 24 hours	Please provide scientific justification for prolonged food deprivation.	

# □ Fluid Manipulation in the form of restriction or regulation (fluid manipulation for clinical reasons such as pre-surgical fasting need not be included in the protocol)

1. Define the proposed fluid manipulation in the table below:

Species	Length of time (Hrs.) it will occur in a single occurrence	Minimum period between manipulations	Explain why the manipulation is necessary to meet the research goals

- 2. Please check yes to provide assurance that animals will be closely monitored to ensure the fluid manipulation will not negatively impact animal welfare. Consequently, body weights will be recorded at least weekly and/or more often for animals under extreme restrictions. [] Yes [] No, please explain.
- 3. Fluid manipulations may negatively impact animal welfare. Consequently, the consideration of potential adverse consequences, and the identification of criteria for the removal (i.e., the humane endpoints) of an animal from the study is necessary. Removal from the study could either be euthanasia, the return to a normal fluid frequency, or another appropriate intervention. Do you expect the fluid restriction to negatively impact animal welfare? [] Yes, complete the table below [] No

Species	Criteria that will be used to remove an animal from the study	Please indicate, yes, to confirm that the euthanasia, final disposition section of this protocol will be applied when removing an animal from this study. (If no, explain)

4. Fluid deprivation for periods exceeding 24 hours can result in prolonged distress for the animals. Will any animals be deprived of fluid for more than 24 hours?
[] Yes, complete the table below [] No

Species	How many animals will be fluid deprived for more than 24 hours	Please provide scientific justification for prolonged fluid deprivation.	

#### □ Samples will be collected from research animals.

- □ Samples will be collected from the animal only after euthanasia.
- □ A non-invasive technique is used to collect, for example, saliva, fecal, urine, hair, etc. samples that will be used for any reason included, e.g., genotyping.
- □ Blood samples will be collected (Check all that apply)

- 1. Please check, yes, to provide assurance that a maximum of 1% of body weight in blood (i.e., 1 ml of blood per one hundred grams of body weight) will be collected in a 14-day (or less) period. *[] yes, [] no, explain.*
- 2. For mice or rats, please check, yes, to provide assurance that the route of blood collection will be lateral, tail and/or saphenous vein, and/or by tail incision, and/or submandibular [] yes, [] no, define and explain the alternate method and why it is necessary.
- 3. Please define and describe how blood will be collected (other than mice or rats)

□ Tissue biopsies will be collected (Check all that apply)

- Biopsies will be collected to genotype animals.
  - 1. If you are using mice and rats, please check yes to confirm that a tail biopsy up to 5mm in length will be collected from a mouse or rat that is less than 21 days of age, or an ear punch for animals exceeding 21 days of age. *[] yes, [] no, explain.*
  - 2. If you are using fish, please check yes to confirm that a sample of the caudal fin will be collected. *[] yes, [] no, explain.*
  - 3. If using any other species, please indicate the type of tissue to be collected, how it will be collected, and reason for using this method.
- Biopsies, for reasons other than genotyping, will be collected. *Please complete the table below.*

Species	Type of Biopsy (e.g., skin, type of organ)	Purpose for and use of the Biopsied tissue

• Is the biopsy collection expected to cause more than momentary pain or distress? [] Yes, please complete the table below, [] No

Species	How many Animals	Anesthetic or Analgesic	Dose	Frequency of application (e.g., every 12 hrs.)

#### □ Behavior Studies will be conducted.

- 1. Please check "Yes" to provide assurance that when behavior studies are performed the animals will be observed (in person or via live video feed) throughout the entire procedure and that they cannot escape the behavior apparatus. [] yes, [] no, explain.
- 2. Will any of the behavior studies to be performed under this protocol result in more than momentary pain or distress for the animals?

#### [] yes, complete the questions below [] no

Species	Name of behavior test	Brief description of the behavior test

a. Animals experiencing more that momentary pain or distress because of a behavior study should receive appropriate interventions (e.g., sedatives) to mitigate the negative effects of the study. *Please complete the table below* 

Species	Total Animals	Name of the Behavior Test	Will animals receive pain/distress pain relieving medications (Yes/No)	Drug Provided	Dose	Scientific Justification if medication is NOT being provided

b. Behavior studies resulting in more than momentary pain or distress may negatively impact animal welfare. Consequently, the identification of criteria for the removal (i.e., the humane endpoints) of an animal from the study is necessary. Removal from the study could either be euthanasia, stop the behavior study, or another appropriate intervention.

Species	Name of behavior test	The criteria to be used for removing an animal from the behavior test

#### □ Breeding

- Investigators should establish breeding colonies based on need and employ carefully designed breeding strategies and accurate genotype assessments to reduce the number of animals used and minimize the generation of unneeded animals. Please check "Yes" to provide assurance that the unnecessary breeding of animals will not occur.
   *[] yes, [] no, explain.*
- 2. For mice and rats, please check "Yes" to provide assurance that standard cage densities (e.g., two adult mice and one litter in a standard cage) will not be exceeded and that animals will be weaned no later than 21 days after birth. [] yes, [] no, explain.

#### **Tumor induction and growth**

- 1. How will tumors be induced? (Check all that apply)
  - $\Box$  Chemically or drug induced.
  - $\Box$  Spontaneous/phenotype
  - □ Injection or implantation of cells or tissues
  - $\Box$  Other,
- 2. Humane experimental endpoints (Check all that apply)
  - □ Mice, Visible Tumors: The animal will be euthanized if the diameter of a tumor exceeds 2cm, or if the tumor ulcerates greater than ½ its surface area, or the tumor develops in an area that impairs normal movement/physiologic behavior.
  - □ Rat, Visible Tumors: The animal will be euthanized if the diameter of a tumor exceeds 4cm, or if the tumor ulcerates greater than ½ its surface area, or the tumor develops in an area that impairs normal movement/physiologic behavior.
  - □ Other species, Visible Tumors: please describe the criteria that will be followed to determine when the animal will be euthanized.
  - □ All species, Non-Visible Tumors: please describe the criteria that will be followed to determine when the animal will be euthanized.

#### □ Other procedures (e.g., Animal identification processes, Trauma, Wound or Burn, Induction of Paralysis), complete the series of questions below for each additional procedure.

In the table below, please provide the name of and a written technical description of those procedures <u>not</u> already described in the protocol above.

Procedure one Name	Species	Briefly describe the procedure and how it will be performed
Gavage (Example)	Mice	Gavage will be used to administer orally a therapeutic agent ABC to evaluate animals. To perform gavage, the therapeutic will be loaded into a syringe, for example, a cannula or tube affixed to the syringe will be carefully placed into the esophagus of the animal and the substance will be carefully delivered directly into the animal's stomach.

1. Will this procedure cause more than momentary pain or distress to the animal?

#### [] Yes, complete the table (Q2) below [] No, Add any additional procedures.

2. Please check yes to provide assurance that analgesia (both pre-emptive and postoperative) will be used for this procedure.

#### [] Yes, complete the table below [] No, please explain in the table below.

Analgesia	Pre-emptive, Post- Procedure, Other	Dose	Total days post Procedure

Procedure two Name	Species	Briefly describe the procedure and how it will be performed	
		The hind leg of the animal will be fractured using blunt force trauma.	
Limb Fracture	Dabbit	To perform the procedure, a conventional guillotine will be weighed	
(example)	παυυπ	and designed to ensure bone fractured without external skin wounds.	
		(Journal of Orthopedic Research, 1984).	

- 1. Will this procedure cause more than momentary pain or distress to the animal?
   [] Yes, complete the table (Q2) below
   [] No, Add any additional procedures.
- 2. Please check yes to provide assurance that analgesia (both pre-emptive and postoperative) will be used for this procedure.

[] Yes, complete the table below [] No, please explain in the table below.

Analgesia	Pre-emptive, Post- Procedure, Other	Dose	Total days post Procedure

#### Humane and Experimental Endpoints (IACUC Section)

1. Will any of the procedures listed in this protocol result in more than momentary pain or distress for the animals? *[] yes, complete the tables below [] no* 

The <u>experimental endpoint</u> of a study occurs when the scientific aims and objectives of the project have been reached. The <u>humane endpoint</u> is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. In some cases, the experimental and humane endpoints must be intricately linked to achieve the project goal while minimizing the pain and distress an animal endures. Developing the project endpoints should be done in consultation with a veterinarian.

Consequently, the identification of criteria for the removal (i.e., the humane endpoints) of an animal from the study is necessary. Removal from the study could either be euthanasia, stop the behavior study, or another appropriate intervention.

Procedure:	
Species:	
Experimental Endpoint:	
Adverse consequences, and the criteria that will be used to remove an animal from the study	
Indicate, yes, confirming the final disposition section of this protocol will be applied when removing an animal from the study. (If no, explain)	

#### □ **Field Studies**

- 1. Briefly describe the activities and procedures that will be conducted in the field, the wild species being utilized, and whether the proposed procedures are expected to influence (e.g., be invasive or change their behavior) the long-term behavior of the animals.
- 2. Please check "Yes" to provide assurance that all required local, state, or federal permits will be acquired prior to the initiation of the animal activities, and that all wildlife regulations will be followed. [] Yes [] No, please explain.
- 3. If not previously identified and described, please identify, and describe the procedures that will be performed in the table below.

Procedure Name	Species	Procedure Description

4. Please check yes, to provide assurance that no wild animals will be housed as part of this study for periods exceeding 12 hours. [] Yes [] No, complete the table below.

Species	Maximum period animals will be housed	Please enter yes to provide assurance that your husbandry protocol, associated procedures, and records, have been reviewed and approved by the AV.

# A formal search for alternatives to Painful and Distressful, which is only applicable to USDA Covered Species (e.g., all mammals except for laboratory mice, rats and birds not bred for research) must be performed.

According to the Animal Welfare Act Regulations, any procedure that will cause more than slight or momentary pain or distress (Categories D and E), requires a literature search to determine if other methods are available for use that would reduce or eliminate the pain and/or distress associated with the procedure. A literature search should consider the following questions: (1) could proposed painful procedures be refined/replaced to reduce the level of pain or distress (a less invasive technique or establishing humane endpoints); (2) are non-animal models available; and (3) could the study be conducted on a fewer sentient species such as invertebrates?

Complete a literature search for <u>each</u> painful procedure using the template below.

Specific Painful Procedure		
Affected Species	Total affected	Category D or E
Years covered by the search	Date of Search	
Sources of the literature search (at least two sources)		
Key words and search terms		
Summary of your search		
that confirms no acceptable		
alternatives were found		

Specific Painful Procedure		
Affected Species	Total affected	Category D or E
Years covered by the search	Date of Search	
Sources of the literature search (at least two sources)		
Key words and search terms		
Summary of your search that confirms no acceptable alternatives were found		