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MEMORANDUM

TO: Principal Investigators

FROM: TCNJ Institutional Animal Care and Use Committee

DATE: July 18th , 2021

RE: *Animal Care and Use Protocol* (ACUP) form

The Institutional Animal Care and Use Committee (IACUC) is responsible for ensuring that any activity at The College of New Jersey that involves the use of vertebrate animals for teaching, research and demonstration purposes meets all applicable federal and state regulatory requirements. As part of these legal compliance requirements, the IACUC must review and approve each study using vertebrate animals.

The ACUP and instructions for its completion are available on the IACUC website at http://www.tcnj.edu/~iacuc. To avoid unnecessary delays, please ensure that you have completed the ACUP form fully and accurately prior to submission.

If you have questions about completing and submitting the ACUP form, please contact the IACUC Chair who will direct you to an appropriate member of the IACUC for additional assistance.

Thank you for your cooperation.

INSTRUCTIONS FOR COMPLETING THE ANIMAL CARE AND USE PROTOCOL (ACUP) FORM

A. General Guidelines

1. Please read and carefully follow all instructions.

2. When submitting an ACUP form, use of the following checklist (do not submit) will help avoid unnecessary delays during the review process:

☐ In addition to providing a reference list in support of the proposed research, a literature search to identify unnecessary duplication and potential alternatives (including consideration of non-animal models and less distressful/painful procedures) is required. Assistance with this type of search is available from TCNJ library personnel.

☐ For USDA category D or E studies, you must work with the Consulting Veterinarian to plan your protocol. For USDA category B or C studies, an initial discussion with the Consulting Veterinarian is highly recommended.

☐ All staff listed in the protocol must have completed the Animal Care and Use Training Program provided by the Consulting Veterinarian. The Consulting Veterinarian will document this training. Discuss with the Consulting Veterinarian any additional or more specialized training needed to complete the proposed research.

☐ If external funds are used to support the project, attach the Animal Care and Use section of the grant proposal.

☐ Submit the final version of the protocol electronically to the IACUC Chair (iacuc@tcnj.edu) for review and approval by the IACUC.

3. After approval of the submitted ACUP form, you will receive an IACUC approval number. ***No animal may be ordered and no animal study can begin without an approved IACUC number.*** Approval by the IACUC in no way obligates the IACUC or The College of New Jersey to guarantee space, animals and/or equipment to conduct the research.

4. Direct any questions concerning animal care, sedation, euthanasia, etc. to the Consulting Veterinarian: Dr. Lauren Bright, lab440@ored.rutgers.edu

5. Direct any material for review, as well as any questions regarding the policies and procedures of the IACUC, to the IACUC Chair:

Dr. Jeffery T. Erickson
Biology Department
Phone: (609) 771-2673
Email: iacuc@tcnj.edu

B. Animals Covered

Any live vertebrate animals used by faculty, students and staff at The College of New Jersey for research, teaching or demonstration purposes.

C. Animal Use Classification Categories

USDA category B: Animals being bred or held for use in teaching, testing, experiments, or surgery but have not yet been used for such purposes. In addition, wild animals that are observed without capture are in category B.

This category includes, but is not limited to breeding colonies whose individuals do not need genotyping using tissue or fluid samples collected from them.

USDA category C: Animals used for teaching, research, experiments or testing purposes in which no pain or distress, or only momentary or slight pain or distress is experienced and for which no use of pain-relieving drugs is necessary. This category includes AVMA-approved humane euthanasia procedures if no other procedures performed put the animals in a higher pain/distress category.

This category includes, but is not limited to, routine physical exams; holding, measuring, or weighing animals; observation of animal behavior in a controlled setting; performing behavioral observations or studies on awake animals with short-term stressful restraint; chemical sedation or anesthesia for restraint purposes only; feeding studies that do not result in clinical health problems; subjecting animals to short periods or overnight food and water deprivation; blood sampling; injecting non-toxic material; injections, fluid collection, or catheter implantation in superficial vessels with no anesthesia; oral administration; gavage; procuring tissue using approved methods of euthanasia that induce rapid unconsciousness; animal identification procedures (tattooing, ear notching, ear punching) that do not require anesthesia; tail sampling in rodents if no anesthesia required; breeding colonies whose members have fluid samples or tissue samples collected for the purpose of determining genotype; live trapping.

USDA category D: requires the use of appropriate anesthetic, analgesic, or tranquilizing drugs. Animals in this category experience more than slight pain and/or discomfort, but the necessary treatments to alleviate the symptoms are available and provided, or the animals are euthanatized. Involvement of trained technicians, scientists, and veterinarians is critical to minimize this pain.

This category includes, but is not limited to, surgical procedures performed under anesthesia, with or without recovery; procedures with recovery that adhere to acceptable veterinary practices, i.e. post-operative analgesia, fluid therapy and required veterinary post-operative care; studies that induce disease or toxicity, such that when clinical symptoms begin to appear, the animals are treated or euthanatized; cardiac blood collection; exsanguination under anesthesia; terminal perfusion.

Comment: Animals used in Category D studies should not show signs of prolonged clinical distress, such as behavioral abnormalities, lack of grooming, dehydration, abnormal vocalization, prolonged anorexia, self-mutilation, or increased signs of infectious processes (peritonitis, pneumonia, diarrhea, encephalitis, etc.). If these clinical abnormalities develop and cannot be alleviated, this will be considered a Category E study unless the animals are euthanatized with minimal delay.

USDA category E: Animals used for teaching, research, experiments, surgery or testing purposes in which the degree of accompanying pain or distress to the animals, and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs are not used due to an adverse effect on the procedures, results, or interpretation of the teaching, research, experiments, surgery or tests. Active involvement of trained technicians, scientists, and veterinarians is required to oversee studies in category E.

This category includes, but is not limited to, prolonged (several hours or more) physical restraint; prolonged deprivation of environmental needs (e.g. maternal care); forced exposure to aggressive behavior or predator-prey interactions; procedures that alter perception or motor function; application of noxious stimuli from which escape is

impossible; exposure to noxious stimuli or agents whose effects are unknown; foot pad or intraperitoneal injections of Freund's complete adjuvant; the use of muscle relaxants or paralytics during surgery without concurrent use of anesthetics; post-operative discomfort that is not treated with analgesics; procedures that produce pain without the use of anesthetics; and induction of aggressive behavior leading to mutilation; toxicity testing where death is the end-point; induction of diseases where affected animals are permitted to succumb rather than be treated therapeutically; severe burn or trauma infliction on unanesthetized animals; using a euthanasia method not approved by the AVMA or the TCNJ IACUC; and exposure to lethal or high doses of x-irradiation or the use of tumor implants/hybridomas.

Comment: Category E studies present an explicit responsibility on the principal investigator to explore alternative methods before proceeding with the study. Before the IACUC can review and approve these projects, the scientific justification must be presented clearly.

D. Preferred Terminology

The IACUC encourages PIs to utilize the following list of preferred terminology when preparing an ACUP. A definition should precede the use of any abbreviations or acronyms, when first used.

Animal facility:	Any of the College facilities used to house animals, such as the 3rd Floor animal facility in the Biology Building. Use in place of ***animal house*** or ***animal room***.
Certification:	Refers to completion of appropriate components of the required IACUC certification program that provides training in the care and use of vertebrate animals at The College of New Jersey. Use in place of ***training***.
Drugs:	Generic names should be used (e.g. sodium pentobarbital rather than nembutal).
Euthanize or Euthanatize:	Use when referring to the humane death of an animal. Use in place of ***kill*** or ***sacrifice***.

E. Instructions by Section

Read all instructions and complete all sections. Do not leave any question unanswered. If any section does not pertain to your study, so state with "N/A". Use terminology and phrasing that is understandable to a lay audience. Define all abbreviations or acronyms at first use. Wherever possible, avoid the use of scientific jargon.

Incomplete or handwritten forms will delay the review of the protocol.

1.a. Application Summary

Title of Protocol: Self-explanatory.

Principal Investigator: Self-explanatory.

Brief Summary of Protocol in Layperson's Terms: Provide a few short paragraphs containing a brief overview of the study/experiment in non-technical terms.

Research Category: Check all that apply. Use the descriptions in the ACUP instructions as a guide.

Surgery? Indicate whether surgical procedures will be used in the protocol. Check Yes or No.

ACUP Type: Self-explanatory. Check all that apply.

Submission Type: If the submission is a triennial review of an existing ACUP with no substantial changes to the procedures used, or an amendment to a current ACUP, provide the IACUC approval number of the previously approved ACUP. All revisions, amendments or changes to a previously approved ACUP, must be highlighted **in bold**. Failure to do so will result in the proposal being returned to you with an associated delay in the review process.

Permits: Self-explanatory. Please note that if permits are required, copies of current and valid permits must be submitted with the completed ACUP.

Funding Source: Check all that apply. If the ACUP will be funded externally, provide the funder's name and grant number, if any. Attach the Animal Care and Use section of the grant proposal to the ACUP submission.

1.b. Application Data

Rationale/Objectives: Provide a detailed description of the objectives of the proposed studies. What is the rationale for the study? What new information will result from the study and what is its importance to the advancement of science? What possible benefits may accrue for the general public from the proposed research? Avoid excessive jargon so that reviewers who are unfamiliar with your research area can gain an appreciation of the study. Define all technical terms in your description. Provide the definition of any acronyms on first use. **For Teaching ACUPs:** Justify the use of animals by the students. How will students who do not wish to participate in the animal procedures satisfy the course requirements? Justify fully why alternate methods of instruction, such as demonstrations or videotapes, cannot be used.

Protocol: Provide details of all experimental procedure(s) as they pertain to the care and use of vertebrate animals, from the time an animal enters the study, until the time the animal is euthanatized or otherwise removed from the study. The IACUC must be able to determine that the study is valid scientifically and not a needless duplication of previous work; therefore, a detailed description of the scientific protocol with appropriate citations is necessary. The use of sub-headings in this section can increase focus and clarity.

Time in Study: Include a statement as to the length of time each animal will typically be in the study and housed in the TCNJ Animal Facility. Different groups of animals may be in the study for different lengths of time.

2.a. Personnel

Principal Investigator: The principal investigator (PI) is the person who assumes the final responsibility for the animals used in a study. Provide all requested information.

All Other Personnel Directly Involved in Project: List all other personnel directly involved in the protocol. Personnel classification includes co-investigator, technician, research assistant, student researcher, and instructor (for teaching ACUPs). Include both a work phone and emergency phone number. If the work and emergency phone numbers are the same, include the number in both spaces provided. If the protocol involves survival

surgical procedures, identify and list a Post-Operative Care Provider with an emergency contact number (***this is required***).

If the personnel list changes (e.g., new students joining the research team or new instructors teaching in the course) after approval of the protocol submit an amendment to the IACUC, provide the names of new personnel and their qualifications.

2.b. Personnel - Training

Research ACUPs: IACUC certification is required for all personnel involved in research activities that involve the use of vertebrate animals. State the training and experience of each person listed in **Section 2.a. Personnel**. List who will be responsible for each procedure including post-operative care and medical record keeping, if needed. Each person listed must receive initial training through the IACUC prior to working with vertebrate animals. The Consulting Veterinarian documents this training. In addition, when applicable, each person must receive any specialized training that may be required to perform the research. The PI or other person in charge of the training must document this training.

Teaching ACUPs: IACUC certification is required for all personnel involved in teaching activities that involve the use of vertebrate animals. State the training and experience of each instructor listed in **Section 2.a. Personnel** who participates in these teaching activities. Describe clearly the training that students will receive concerning the ethical care and use of the vertebrate animals used in the teaching activities and provide a brief synopsis or outline of any training material or procedures that will be used that are provided to the students.

3.a. Species and Study Type – Animal Numbers

Name of Species/Strain/Breed/Gender and Source: Common names (rat, rabbit, dog, etc.) will suffice. Indicate the vendor or source for each species used.

Number of Animals: Provide the number of animals required for each category (see Section C) and provide the total number of animals per species requested. Do not provide a range (e.g. 10 to 15).

The Animal Welfare Act Mandates Involvement of a Veterinarian in the Planning of Category D and E Studies: Provide the name of the veterinarian consulted and the date of the consultation for Category D and/or Category E studies. For Category E studies, there must be scientific justification for not using anesthetic, analgesic, or tranquilizing drugs when animals experience more than momentary/slight pain or distress.

3.b. Species and Study Type – Animal Number Justification

Justify the *maximum* number of animals required, indicating how that number was determined. Use experimental design considerations rather than workload to determine this number. Include a table or flow chart showing treatment groups and number per group if this helps clarify and justify the number of animals requested. **The total number of animals of each species must be identical to the number listed in Section 3.a. Species and Study Type – Animal Numbers.**

If the required number of animals changes after the protocol is approved, submit an amendment to the IACUC with an explanation and justification for the change.

4. Housing and Enrichment

Housing: Appropriate social interactions among members of the same species are essential to normal development and well-being. Social species should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons, veterinary related concerns or because of social incompatibility. Single housing should be limited to the minimum period necessary. Complete the checklist, and for each box checked "yes", describe the situation and provide justification as to why the animals cannot be housed in pairs or groups.

Enrichment: The primary aim of environmental enrichment is to enhance animal well-being. Providing stimuli, structures, and resources that facilitate the expression of species-specific behaviors can accomplish this aim. However, in some instances providing environmental enrichment may introduce an unwanted variable in a research study. Species-specific enrichment materials (e.g. nesting materials for mice, shelters for rodents, shelters and climbing structures for snakes, etc.) should be utilized unless there is an experimental reason not to provide an enriched environment. Check the "yes" or "no" box. If the "yes" box is checked, describe the form of the enrichment. If the "no" box is checked, provide a justification for the lack of environmental enrichment.

5.a. Hazards

Indicate hazards to humans and/or animals by checking all appropriate boxes. For all boxes checked, briefly describe the specific nature of the hazard in the space provided.

5.b. Hazards – Preventative Measures

For all hazards listed in **Section 5.a. Hazards**, provide a detailed description of environmental health and safety concerns and all procedures used to protect animals and/or humans from the hazard.

6. Checklist

Consider each item carefully and check "yes" to all that apply or "no" if they do not apply. For every item checked "yes", provide a description in space provided. Please note that written approval to use Freund's Adjuvant must be obtained from the Consulting Veterinarian. The approval must be attached to the completed ACUP at the time of submission.

7. Dosing

If drug administration is included in the protocol, complete this table, providing all requested information.

Drug: List the generic drug name (e.g. sodium pentobarbital rather than Nembutal)

Dose: Provide on a body weight basis, if possible, and indicate drug units.

Route: Indicate the route of drug administration (e.g., intramuscularly, intraperitoneally, intravascularly, etc.).

Maximum volume: Indicate the maximum volume used for injection (µL, mL, etc.).

Frequency: Indicate the frequency of drug administration.

8. Bleeding

If blood withdrawal is included in the protocol, complete this table, providing all requested information.

9. Anesthesia, Analgesia, Tranquilizers and Paralytics

If anesthesia, analgesia, tranquilizers or paralytics will be used in the protocol, complete this table, providing all requested information.

Induction Dose: Provide in mg/kg body weight or percent gas.
Maintenance Dose: Provide in mg/kg body weight or percent gas.
Route: Indicate the route of administration (e.g. inhalant, intraperitoneal injection, etc.)
Administered: Indicate whether the agent will be administered pre-, peri- and/or post-operatively.

10. Surgery

If surgical procedures are included in the protocol, check either "yes" or "no". If yes, check all additional boxes that apply and provide a detailed description of each procedure in the space provided (**Description of Surgical Procedures**), including preparation of the animal, instruments used, precautions taken to minimize infection and post-operative procedures.

11. Euthanasia

Indicate all methods of euthanasia used in the protocol by checking all boxes that apply. For any method that is not listed, check the box marked "Other" and list where requested. Please refer to the most recent edition of the AVMA Guidelines for the Euthanasia of Animals to ensure that you are using the most appropriate method of euthanasia for your species and research needs. You may find the AVMA Guidelines at:
https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
For any agent used in the protocol other than carbon dioxide gas, provide all requested information in the table provided

Species: Self-explanatory
Agent: Provide the generic name
Induction Dose: Provide in mg/kg or percent gas
Route: Indicate the route of administration (e.g. inhalant, intraperitoneal injection, etc.)
Method: State the method of euthanasia used. Examples include cardiectomy, exsanguination, cervical dislocation and decapitation. If cervical dislocation or decapitation is used, provide a justification in the space provided.

Justification of cervical dislocation and decapitation: Self-explanatory

Euthanasia for sick animals: Describe how animals will be euthanatized in case of illness.

Final Disposition of Animals: Describe how and when animals will be removed from the study. This may include a humane (terminal) endpoint.

12.a. Unnecessary Duplications and Alternatives

Indicate which databases and other sources have been searched to ensure that the proposed experiments do not unnecessarily duplicate previous experiments, cause undue stress or pain, and that less painful or distressful alternatives have been considered and are not available. USDA Policy #12 recommends that "the databases searched, the date of the search, and the years covered by the search" be included. Provide the range of years covered with a defined latest year rather than "to present". If this is a Category D or Category E ACUP, provide the parameters (keywords, title words, etc.) used for the search. Assistance with an alternatives search is available from the TCNJ library. Indicate whether the PI attends scientific meetings, is a journal reviewer, and/or has consulted with an expert not affiliated with TCNJ.

12.b. Unnecessary Duplications and Alternatives – Justifications

Justify why an animal model is required and why computer/mathematical models, cell or organ culture methods, and/or other alternative models are not appropriate. Provide written assurance that the activities do not unnecessarily duplicate previous experiments, and that the investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals.

Describe any alternatives that were incorporated into the protocol. The concepts of alternatives concerning the use of animals is built on the three R's: Replacement, Reduction, and Refinement. This includes the replacement of animals used in teaching or research with alternatives when possible, the reduction in the number of animals used, and the refinement of animal use to include new approaches that are less invasive, painful and/or distressful to the research animal.

Justify why the species requested is/are the most appropriate. Self-explanatory

Describe any steps that have been taken to reduce the number of animals required to complete the proposed studies. Self-explanatory.

Briefly describe steps that will be taken to minimize pain and distress (e.g. pre-emptive use of analgesics, refinement of invasive techniques, etc.). Scientific justification is required for any surgical procedures without the use of analgesics, anesthetics or tranquilizing drugs (USDA Category E).

13. Laboratory and Procedure Room Locations

List all laboratory and procedure room locations in the table provided and check all boxes that apply. (SS) survival surgery; (NSS) non-survival surgery; (>12hrs) *in vivo* research for more than 12 hours outside the Animal Facility; (Other) other procedures (e.g. tissue harvesting or injections). All SS, NSS and >12hrs labs will be inspected by the IACUC prior to study initiation with follow-up semi-annual inspections.

In the space below the table, briefly provide justification for housing animals outside of the Animal Facility on the third floor of the Biology Building.

In the space below the table, identify which procedures will be conducted in an investigator's research laboratory and justify why these procedures cannot be conducted in the TCNJ Animal Facility.

14. References

Provide full citations for any publications referenced in the ACUP.