

STANDARD PROCEDURE
for
ANIMAL CARE and USE PROCEDURES
REVIEW

All animal care and use procedures (ACUPs) involving animals covered under the regulations of the Animal Welfare Act must be approved by the Institute Animal Care and Use Committee (IACUC) **before** such procedures commence. All submissions, reviews and retention of documents relating to ACUPs will be handled in such a manner as to comply with applicable federal regulations. The standard procedures associated with the review of Animal Care and Use Procedures are given below:

I. SUBMISSIONS AND VETERINARY CONSULTATION

All animal care and use procedures at Rose-Hulman Institute of Technology are to be documented on an ACUP Form (see Attachment A). The person responsible for the submission of the written procedures is termed the Principal Investigator (PI). The PI is defined as *"..... an employee of Rose-Hulman Institute of Technology, or other person associated with Rose-Hulman, responsible for a proposal to conduct experimental research or to conduct an experiment or demonstration for teaching purposes and for design and implementation of such experiments involving animals"*. The form requests the information necessary for review by the Institute Animal Care and Use Committee (IACUC).

The law requires that an Attending Veterinarian (AV) or his/her designee be consulted by the PI when planning procedures in order to provide guidance in matters of animal medicine/technology and information necessary to complete immobilization, anesthesia, analgesia, tranquilization, euthanasia, and adequate pre-procedural and post-procedural care in accordance with current established veterinary medical and nursing procedures. The PI is to consult with the attending veterinarian prior to completing the form.

Once the form is completed, it will be recorded and forwarded to the IACUC Secretary for final review/consultation and assigned an ACUP number.

II. ACUP REVIEW by the IACUC

The procedures and associated information contained within the completed ACUP form are to be reviewed by the full IACUC or by at least two members of the IACUC acting as a subcommittee. Many ACUP reviews will be performed by subcommittee.

USDA regulations require that all members of the IACUC have the opportunity to request and receive a full IACUC review of any ACUP. Consequently, each ACUP will be provided to all IACUC members five (5) working days in advance of the Subcommittee review. Unless one of the IACUC members requests full committee review, the ACUP review will be handled by the subcommittee. If there is a request for full IACUC review, arrangements for that review will be made by the IACUC Chairman or his/her designee.

There will be an exception to the five day notice period for urgent ACUPs to be reviewed by the IACUC. In such cases, a telephone poll will be conducted to determine whether any committee member desires full IACUC review.

All ACUPs will be reviewed by either the IACUC or its subcommittee, and reviews will be conducted in the following manner:

1. The Chairman or his/her designee will specify a primary and secondary reviewer for each ACUP. All IACUC members will receive a copy of the ACUP Abstract with reviewers designated on the form.

2. Primary and secondary reviewers will receive a copy of the ACUP at least five working days in advance of any IACUC or subcommittee review; in the case of the expedited review a copy of the ACUP may be provided with less lead time. It is the reviewers' responsibility to evaluate the procedures and other information, including making direct contact with the PI for explanation of procedures unfamiliar to the reviewer or for other clarifying information.
3. The reviewers will decide the ACUP's compliance with USDA regulations and provide comments/approval to the IACUC Chairman. Such approval shall, at the discretion of the Chairman, extend only until the IACUC has an opportunity to review the ACUP. The reviewers shall return the reviewer's minutes form to the IACUC Chairman who will notify the PI of the IACUC action and record the action in the IACUC record.

The vote may result in the following:

- i) APPROVAL
- ii) WITHHOLD APPROVAL
- iii) REQUIRED MODIFICATIONS
(Returned to PI with list of modifications required for approval.)

The IACUC Chairman or his/her designee is responsible for recording the minutes of the review.

4. In the case of full IACUC review, the primary reviewer is responsible for presenting the ACUP to the IACUC. The ACUP will then be discussed and upon conclusion of the discussion, a vote of IACUC members in attendance is taken. The PI will not be present for the discussion and the vote of approval, although he/she may make themselves available to answer questions.

III. NOTIFICATION TO PRINCIPAL INVESTIGATOR

The Chairman is responsible for notifying the PI in writing of the action taken by the reviewing body as soon as reasonably possible. If the ACUP is approved, the notification will include the ACUP number that has been assigned.

IV. RESUBMISSION OF ACUPS

A. Required Modifications

ACUPs requiring modification will be returned to the PI to be amended and resubmitted into the ACUP review process. The review and notification procedure are the same as mentioned previously.

B. Annual Review of ACUPS

All ACUPs will undergo periodic review, at least annually, after their initial submission. The IACUC will establish the review cycle for each ACUP and at its discretion may require more frequent review of some ACUPs. In either case the

PI will be notified of the review requirement. He/she will then be responsible for updating the ACUP's information and submitting for review as outlined above.

C. Substantive Changes in ACUP

The ACUP is specific and unique in a number of ways including animal species, persons performing procedures, techniques to be used, method of euthanasia (if required), method of anesthesia (if required), etc. If there are changes in these or any other substantive information within the ACUP, the PI is required to modify the ACUP and submit it as a new procedure.

V. IACUC CONSIDERATION

A majority of the IACUC will hold periodic meetings to consider the actions of the ACUP Subcommittee. All members at this session of the IACUC will have an additional opportunity to ask questions concerning individual ACUPs, and the Subcommittee will discuss significant issues that may have arisen during the ACUP review process. At the close of discussions the IACUC will affirm or disaffirm the actions of the ACUP Subcommittee. If the IACUC decisions affect individual ACUPs, the Chairman of the IACUC will notify the appropriate PI of the need to update or amend the ACUP in question.

List of Attachments

Attachment A: Animal Care and Use Procedure (ACUP) Form

Attachment B: Review and Evaluation Form

Attachment C: Notification Form

ATTACHMENT A

ANIMAL CARE AND USE PROCEDURES (ACUP) FORM

Purpose of Proposed Research or Class Experiment/Demonstration

Briefly explain in language understandable to a layperson the aim of the research or teaching experiment/demonstration proposed, including why the research or teaching experiment/demonstration is important to human or animal health, the advancement of knowledge, or the good of society.

The purpose of this experiment is to examine excitation-contraction coupling in skeletal muscle using the isolated frog gastrocnemius muscle as a model system. Students are asked to isolate the femur, gastrocnemius muscle, and associated motor nerve of a frog that has been doubly pithed. These structures are removed and the femur is held with a clamp with the muscle hanging freely. The free end of the muscle is attached to a force transducer using a suture. The motor nerve that drives the muscle is then stimulated, causing contraction of the muscle. The force of this contraction is measured by the force transducer.

Rationale for Animal Use

- 1) Explain your rationale for animal use, including reasons why non-animal models cannot be used.
- 2) Justify the appropriateness of the species selected. [Nb. The species selected should be the lowest possible on the phylogenetic scale.]

The frog model was chosen because the gastrocnemius muscle preparation has properties that make it well-suited for this application. For instance, the anatomy of the frog leg makes it relatively easy for students to isolate and remove the femur/gastrocnemius complex. Also, this muscle is able to generate very high tensions, which makes it relatively easy to record data for the stimulation/response curve. In addition, frogs are relatively inexpensive and easy to keep. It would be possible to do this experiment using a computer simulation, but we feel that this would decrease the effectiveness of the laboratory as a teaching tool. An important aspect of this lab is to give students experience in performing the appropriate dissections, setting up the recording apparatus, and performing some troubleshooting of the system as problems arise. The use of a computer simulation would result in the students missing out on these important experiences.

Experimental Protocol/Time Line

Provide a description of all procedures using animals. For animals undergoing non-survival surgery solely for tissue procurement, a short description of the procedures for obtaining tissue and a brief description of the usage planned for the tissues will be adequate. A description of restraint and anesthesia protocols must be provided in sections IV and V, respectively, and non-surgical and surgical procedures must be provided in sections VI and VII, respectively. These details need not be provided here.

Provide a breakdown of the experimental groups and an experimental protocol for all procedures using animals. For animals undergoing survival surgery, multiple manipulations, and/or repeated observation, a time line is necessary. The time line should begin with animal procurement, denote the approximate timing of all important manipulations, and conclude with final disposition.

Animals will be doubly pithed before dissection. Pithing will be done by the professor (Dr. Weiner has significant experience in this technique). Students will be given the doubly-pithed animal and will remove the femur, gastrocnemius muscle, and associated motor nerve. Students then mount the femur in a clamp and connect the other end of the muscle to a force transducer. The motor nerve is connected to a bipolar stimulator. A software based data acquisition program system will control stimulation of the nerve and recording the output of the force transducer.

In the recent past, the physiology course at Rose-Hulman has had an enrollment of roughly 10 students. These ten students will be divided into 4 laboratory groups. Two lab groups will share a single animal, although there is a distinct possibility that there will be errors in dissection that would require the use of an extra animal. Therefore, we anticipate that we will require 3 animals per lab section. One or two additional animal will be used by the instructors to test the experimental setup prior to the first time teaching this laboratory. Therefore for the first year that this laboratory is taught, it is expected that we will use at most 5 frogs for this teaching laboratory.

The number of students taking physiology, and therefore the number of animals required for this lab, is expected to grow significantly in the next few years due to maturation of the Applied Biology B.S. program and initiation of the Biomedical Engineering B.S. program.

Personnel and Qualifications

Regulations issued under the recently amended Animal Welfare Act require that "Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures..." In addition to the responsibility of the Principal Investigator to assure that those individuals conducting such procedures are properly trained and qualified, the Institute Animal Care and use Committee is required to evaluate the qualifications provided by the Principal Investigator in the course of ACUP review.

Descriptions of qualifications must include 1) formal education, 2) relevant general experience in the performance of procedures in the species studied, and, as applicable, 3) specific training and experience in the performance of non-routine, invasive, and surgical procedures in the species studied.

Research protocols should include only the names of the Principal Investigator and those individuals actually performing animal care and use procedures. Teaching protocols should include only the names of the Instructor and those employed to assist the instructor during the teaching exercise. Exclude the names and qualifications of those individuals with non-animal support functions associated with the research/teaching protocol. Descriptions of training and experience in the performance of specified non-routine, invasive, and surgical procedures, unless they will be performed by a Clinical Veterinarian, must be included. If training/CV not attached, describe the training and experience of each individual.

CV attached or on file
(include training)

Principal Investigator/Instructor: Jameel Ahmed*

Co-Investigators/Instructors: William Weiner**

Technicians:

Notes:

* Dr. Ahmed has performed animal experiments since 1991

** Dr. Weiner has taught teaching laboratories involving frogs in the past. He has experience in performing the frog double-pithing procedure. .

SECTION II. ANIMALS AND HUSBANDRY REQUIREMENTS

What genus, species, and strain of animal will be used? Leopard Frog (*Rana pipiens*)

What sex distribution is required? male female both either

What will be the approximate age, weight or size of the animals used? adults

What is the maximum number of animals per study? 5

How many control groups are planned? 0

What is the maximum number of treatment groups per study (include controls)? 0

How many animals per treatment group? 0

Is after hours monitoring of animals required? Yes No

If yes, explain the circumstances that necessitate after hours monitoring and who will monitor.

Is a special diet required? No

What is the maximum number of days the diet will be fed?

What, if any, are the anticipated side effects?

Is dietary restriction required?

If yes, what is the anticipated maximum duration (hours) of dietary restriction?

How often will the diet be restricted (frequency)?

Is special housing required? No

If yes, describe housing requirements.

SECTION III. ROUTES OF ADMINISTRATION, TEST SUBSTANCES

Will a test substance be administered? Yes No

If no, go to Section IV.

If yes, indicate all routes of administration:

Describe the dosing regimen, including maximum frequency and duration, for each route of administration:

SECTION IV. RESTRAINT

Will animals be restrained? yes

If any test substance as described in Section III will be administered, will the method of restraint (for test substance administration only) be brief, routine restraint? Yes No

If yes, do not include a description of the restraint technique for test substance administration below.

If no, include a description of the restraint technique to be used for test substance administration below.

Describe the applicable methods of restraint for each procedure. The descriptions should include the restraint methods to be used for procedures such as examination, test substance administration (other than routine manual restraint), anesthesia administration, and euthanasia, as well as for procedures unique to this protocol.

Routine manual restraint will be used during the pithing procedure

Chemical Restraint, non-surgical procedures. List procedure(s), the chemical agent including dose and route of administration, and anticipated duration and frequency.

SECTION V. EUTHANASIA

Will the animals be euthanized? Yes

If no, go to Section VI.

If yes, indicate method(s). If a method employs the use of a drug or chemical, indicate the name of the agent, dose and route of administration.

Double pithing - This is done by holding the animal with its head bent forward, exposing an area called the foramen magnum. A sharp probe is inserted through this opening and into the brain. The brain is mechanically destroyed using sharp movements, immediately destroying all brain centers in the animal. The effectiveness of the pithing can be gaged by checking the corneal reflex. If the eyelid does not close upon touching the cornea, it is clear that the pithing effectively stopped all brain function.

After this initial brain destruction, the probe is then inserted into the spinal cord, disrupting all reflex action within the spinal cord. The effectiveness of destruction of the spinal cord can be tested by examining the tone in leg muscles. Insertion of the probe into the spinal cord should lead to complete contraction of the leg muscles, followed by complete relaxation. Any tone in the leg muscles indicates that there is still some spinal function intact.

Dr. Weiner has significant experience with the pithing technique. Pithing is approved by the AMVA as a humane form of euthanasia for amphibia.

SECTION VI. NON-SURGICAL PROCEDURES

Is it anticipated that any of the non-surgical animal procedures conducted in the course of this study will cause pain or distress, other than that which is momentary or transient?

No

If no, go to section VII

If yes, identify each procedure and any measures taken to alleviate pain or distress pre-procedural, procedural, and post-procedural, including the use of anesthetics, analgesics and tranquilizers, or other measures such as behavioral conditioning.

If yes, and measures will not be taken to alleviate pain or distress, what is the rationale for not alleviating pain or distress?

Retrospective reporting of any unanticipated pain or distress that occurs in study animals for any reason is the responsibility of the Principal Investigator. This applies to surgical as well as non-surgical procedures, and includes painful or distressful effects of test substances. As soon as the animal portion of the study is completed, the Principal Investigator should submit a written report stating (a) the ACUP number, (b) the numbers and species of animals involved, (c) a description of the events resulting in unanticipated pain or distress, and (d) measures taken to prevent future occurrences of such events, and, should such events recur, measures to be taken to alleviate the pain or distress.

SECTION VII. SURGICAL PROCEDURES

Will surgery be performed? No

If no, go to Section VIII.

Who will perform the surgical procedures?

Where will the surgical procedures be performed (building and room/lab numbers)?

Will the surgical procedures be major? or minor?

Will survival surgical procedures be performed?

All survival surgical procedures must be performed using aseptic technique.

If yes, will multiple survival surgical procedures be performed?

If multiple survival surgical procedures will be performed, provide an explanation of why this is necessary.

Will chronic instrumentation be performed?

List dose and route of administration of each agent used as a(n):

Pre-anesthetic:

Anesthetic:

Post-anesthetic:

Will an analgesic be administered post-operatively?

If yes, identify each analgesic to be administered and indicate the dose, route of administration, and frequency of administration.

If post-surgical pain or distress is anticipated, and an analgesic or other appropriate agent will not be administered, explain the necessity of withholding or the contraindications to the use of analgesics or other appropriate agents.

Who will monitor the animals during anesthesia?

Who will provide post-surgical care/monitoring?

Where will the animals recover from anesthesia?

Will any paralytic or neuromuscular blocking agent be administered?

If yes, indicate the agent, dose, and route of administration.

SECTION VIII. EVALUATION OF ALTERNATIVES TO THE USE OF ANIMALS AND PAINFUL PROCEDURES

A. Replacement Alternatives

Describe any alternatives to animal testing and/or painful procedures you considered for this protocol. The investigator is required to consult information sources such as Biological Abstracts, Index Medicus, Medline, Current Research Information Service, the Animal Welfare Information Center (National Agricultural Library), or other sources in an effort to identify possible replacement alternatives. List the information sources consulted and describe any alternatives to animal testing procedures you considered. If existing alternatives are unsuitable, explain. If the use of animal testing is required by regulations, cite the appropriate regulation and/or testing guideline.

There have been software simulations developed for this laboratory exercise. Although these do illustrate the conceptual parts of the laboratory, they are a poor substitute for the hands-on aspects of the laboratory experience. These hands-on aspects include dissection, connection of the muscle to a mechanical recording system, stimulation of a motor nerve using a bipolar electrode, and an exposure to the inherent variability associated with recording from living systems.

B. Reduction Alternatives

Describe measures considered, such as appropriate statistical evaluations, or the use of *in vitro* modeling coupled with an *in vivo* component, that tend to reduce the total number of animals used.

n/a

C. Refinement Alternatives (Minimization of Pain and Distress)

If painful or distressful procedures are a component of your procedure, what measures are taken to employ methods that tend to minimize the degree or duration of pain or distress, other than the administration of anesthetics, analgesics, or tranquilizers, such as 1) the minimization of time of exposure to a painful or distressful procedure or situation, 2) behavioral conditioning or modification, and 3) the use of available spontaneous animal models in lieu of induced animal models?

n/a

SECTION IX. RESEARCH DUPLICATION

(Skip Section IX if ACUP is for teaching purposes.)

Has this protocol been reviewed against data already in the literature to ensure that this study does not unnecessarily duplicate previous experiments? Describe your review.

The responsible Principal Investigator affirms that the study design, for which the above procedures will be performed, represents his/her best efforts for the reduction of numbers of animals used in this research, including the consideration of alternatives to the use of animals, and for the minimization of pain/distress in research animals. Furthermore, the experience of the individuals listed in the qualifications section have, individually or collectively, sufficient education, training, and experience to perform all of their assigned portions of the described animal care and use activities. The Principal Investigator will review and reevaluate the qualifications and training of all personnel involved in these animal care and use activities on at least an annual basis.

Principal Investigator

Date

THIS SECTION TO BE COMPLETED BY VETERINARIAN

The Veterinarian has reviewed the proposed animal care and use procedures and has found them appropriate for this protocol.

Attending Veterinarian

Date

ATTACHMENT B

REVIEW AND EVALUATION FORM

ACUP REVIEW AND EVALUATION RECORD

ACUP No: _____ **Date:** _____

Title: _____

ACUP Review Subcommittee: _____

Primary Reviewer: _____ **Secondary Reviewer:** _____

Subcommittee review and evaluation of this submission included but was not limited to consideration of the following: (all may not apply)

The Principal Investigator responsible for this ACUP has offered written assurance that where applicable:

1. The species selected is considered appropriate for this procedure.
2. The experience and qualifications of the Principal Investigator and Laboratory Assistants are appropriate to perform this activity.
3. Living conditions will be in accordance with existing regulatory standards.
4. Minimum number of animals are to be used consistent with the needs of the procedure described.
5. Painless euthanasia will be provided if needed.
6. Pain, discomfort, and distress will be minimized.
7. More than momentary or slight pain will be appropriately eliminated or minimized or will continue only for the necessary period of time.
8. Animals experiencing chronic or severe unrelieved pain will be euthanized during or immediately at the end of the procedure.
9. Appropriate surgical services and pre- and post-operative veterinary and nursing care will be provided.
10. Multiple survival operative procedures will not be performed unless appropriate justification is provided.
11. A literature search was conducted and non-animal alternatives were either not available or considered not appropriate.
12. This activity does not unnecessarily duplicate previous experiments. (Research only)
13. Medical care is available and will be provided by a qualified veterinarian.

Action Approved

Require Modification(s)
(List)

Approval Withheld

Reviewer

Date

ATTACHMENT C

NOTIFICATION FORM

NOTIFICATION TO INVESTIGATOR OF ACUP REVIEW

To: _____ (*Principal Investigator*)

ACUP No. _____ has been reviewed by the Institutional Animal Care and Use Committee (IACUC), and the following action was taken:

- Approved
- Approval Withheld
- Requires Modification

If this ACUP requires modification(s), this is described below. All changes should be discussed with the primary reviewer listed below, or in case they are unavailable, with the secondary reviewer.

REQUIRED MODIFICATION(S):

Upon modification, please resubmit the completed ACUP. No experiment can be initiated for this ACUP until the Principal Investigator has received written notification of the ACUP's approval.

Signed: _____

Date: _____