

Southern Arkansas University
General IACUC 3 Year Research Protocol Form
Rev.1 Fall 2019

Instructions: This form is required for any vertebrate research conducted at Southern Arkansas University or its campuses. Refer to the IACUC Guide for the care and use of laboratory animals (available online and SAU IACUC) for assistance with writing your protocol. Any protocol questions and form questions can be directed to SAU IACUC members. The completed form can be submitted to the chair of the SAU IACUC. Approved protocols are valid for 3 years from the approval date. Any modifications to an approved protocol can be made via the protocol modification form. Annual review of all protocols is required. An Annual Review Form will be sent to the Principal Investigator approximately 30 days prior to the Annual Review Due Date.

General Information

1. Protocol Title – Teaching with mice (applies to BIOL4244 Advanced Microscopy, BIOL 3373 Neuroscience, BIOL4033 Developmental Biology)
NOTE: This is a teaching application and not a research application.
2. Animal Species (list all that will be covered under this protocol) – Mice and Zebrafish
3. Lay Overview (state the research or teaching goals in two or three sentences that can be understood by a lay person. Please avoid technical terms & acronyms) – A variety of live animals and their tissues are used in biology teaching labs. Up to 60 adult zebrafish (*Danio rerio*) will be kept for breeding and supplying embryos for developmental biology labs and neuroscience labs. Adult mice will be kept to provide tissue for advanced microscopy labs and neuroscience labs.
4. In lay terms, describe how this research or teaching will benefit society, advance knowledge, or benefit human or animal health. – Much of the excitement of being a scientist comes from discovering something new about how the world works. In this part of the course, the students will have the opportunity to carry their own experiments, building on what they learned during the lecture course. Experiments will focus on the mechanisms that pattern the embryo during vertebrate development and how these mechanisms are explored using molecular, cellular, and genetic approaches. Students will learn how to generate a hypothesis, design and carry out experiments to test their hypothesis, and gather data and form conclusions.
5. Study Endpoint for Animals
☒ Acute terminal (animal never awakens from initial procedure)
☐ Survival (how long) for
If survival, How long?

Personnel

Note all faculty and students who will have contact with the animals.

Name	Position	Service (see below)
James Hyde	Instructor	S, In, An, Eu, Hz, DD, HH, Br, BT, NI, Ob, SR

Position: Note if the person is faculty or a student. Also note who is the PI for this protocol.

Service: What is this person trained to do? Surgery (S), Injections (In), Anesthesia (An), Euthanasia (Eu), Hazard Material Administration (Hz), Drug/Diet Administration (DD), Handling/Husbandry (HH), Breeding Management (Br), Blood/Tissue Collection (BT), Non-invasive Testing (NI), Observation (Ob), Surgical Records (SR)

Federally Required Information & Assurances

In accordance with Public Law 89-544 (Animal Welfare Act Amendments of 1970), Public Law 94-279 (Animal Welfare Act Amendments of 1976), Public Law 99-196 (Food Security Act of 1965, Subtitle F – Animal Welfare), Code of Federal Regulations, Title 9, Chapter 1, Subchapter A – Animal Welfare & the Public Health Service Policy on Humane Care & Use of Animals, the Southern Arkansas University Animal Subject Committee is required to obtain the following:

6. Why are living animals required for your study? Why can't you use replacements such as cell culture, computer modeling or other non-animal models? Check all that apply
- ☒ The complexity of the processes being studied cannot be duplicated or modeled in simpler systems
 - ☐ There is not enough information known about the processes being studied to design non-living models
 - ☐ Pre-clinical studies in living animals are required by federal regulations prior to human testing
 - ☒ This is a behavioral, learning, or developmental study

Other:

7. Why is the proposed species the most appropriate? Why can't a less sentient, phylogenetically lower species be used? Check all that apply:

- ☐ A large database exists for this species allowing comparisons with previous data.
- ☐ The anatomy, genetics, physiology or behavior of this species is uniquely suited to the study of proposed.

Describe below.

- ☒ This is the phylogenetically lowest species that provides adequate size, tissue, or anatomy for the proposed study.

☐ This species provides a particularly good model for duplicating the human situation. Describe below.

☐ The results will be directly applicable to the health or care of this species. Describe below.

☒ Previous studies using this species formed the background for this project.

☐ The species has unique features that make it the best choice available for this study. Describe below.

Explanation for any choice where description is required: These are common animal models used in undergraduate level education and have been used in the classes in previous years.

8. Duplication of Research

☒ By checking here, I certify that in planning this experiment I have reviewed the relevant literature (by computer database literature search, use of comprehensive review articles, consultation with Animal Welfare Information center, etc.). Based on the literature, I certify that the activities involving animals described in this protocol do not unnecessarily duplicate previous research. Assure that I will retain my search records for three years past the end of the animal studies & that these search records will be available to Inspectors at any time.

9. Consideration of Alternatives to Painful Procedures

Are you:

☒ Using warm-blooded vertebrate species OTHER THAN rats, mice, or birds.

☒ Proposing procedures that fall into the USDA Pain Category D or E (Causing more than momentary or slight pain or distress)

Animal requirements for the 3 year approval period

10. Strains/Breeds

Breed (Strain/Genotype)	Phenotype/Health Issues	Age (adult, juv. Fetus, etc)
BALB/C mice	none	Adults
Danio rerio (Zebrafish)	none	Adults, Juv., embryos

11. Describe your experimental design: include all procedures, manipulations, timelines, & endpoints. If a procedure, surgery, or collection is performed multiple times, this must be described here. Do not include procedural details here; procedures should be described in the sections below. A reviewer should be able to understand the complete time course of procedures on each animal that you describe in the studies below.

Mice

Mice will be housed only long enough for the current experiment. Only enough mice for the current experiment will be purchased at that time. Mice will be housed according to gender with no more than 4 mice per cage with every attempt to avoid single housing animals.

Either fixed or live tissue will be collected from mice. In the case of live tissue for neuroscience, mice will receive an intraperitoneal injection of urethane. The mouse is decapitated and the brain will be rapidly dissected and used for physiology experiments. If fixed tissue is necessary for studying tissue microstructure, mice will be similarly anesthetized. They are exsanguinated and perfused with saline following anesthesia. Finally, they are perfused with fixatives and the tissues are dissected out for analysis.

Zebrafish Adults

The PI will set up natural matings of adult fish throughout the semester. Note that adult fish can be set up in matings once or twice a week, so the same adult fish can be used many times during the semester. All experiments will be done on the embryos produced, which are too young to fall under IACUC oversight, as they do not yet have backbones.

Adults are the only vertebrates that will be kept and are only used for breeding. At least 2 (5L) tanks are maintained at one time for males and females. Every 5L tank can hold up to 30 adult fish for a total of up to 60 fish. This is important, as many factors (many unknown) affect the egg laying rate of the adult fish. Males and females will be temporarily transferred to smaller breeding tanks as needed. They will only be kept in these tanks for up to 7 days before being returned to the larger population. There should not be any affects from natural matings of the adult fish. If we have any sick fish, they will be removed from their home tank and either treated with appropriate medicines or euthanized to prevent the spread of any disease.

The fish are maintained as breeding stocks, and thus are kept until they are too old to mate and are exhibiting signs of old age. The life span of zebrafish is approximately two years. As fish are approaching this two year mark, decreases in their rate of reproduction, a decrease in their weight ("skinny fish"), and bends in their backbone indicate end of life. These fish are euthanized as below as a humane practice and because elderly fish are more likely to get diseases that could be passed on to other fish in the tanks.

Daily checks will include checks for leaks, filters, running water, and temperature. Nitrites will be checked bi-weekly or as needed. Tank changes and cleaning will be conducted as needed to control algae.

If a fish is visibly sick, (lethargic, skinny, open wounds) it should be removed and euthanized. This information will be recorded in fish logs. To euthanize, aquatic system water will be placed in a 1L tank and dosed with 250-500mg/L Tricaine (MS222) as per the 2020 AVMA guidelines. After immersion for 30 minutes, fish will be decapitated and the carcasses will be disposed of according to department protocols.

12. Fill out this section if your experiments use animals in pain categories C, D, or E. See the Pain Categories document for explanation of each category.

	Number of Animals in Each Category of Pain/Distress			
Group Name	C	D	E	
Mice		40		
Zebrafish		100		
				Total C+D+E
Subtotals		150		150

13. Provide any additional justification for the number of animals requested. How were the sample size, number of groups, & number of repetitions determined? Check all that apply.

☐ Power analyses indicate that the proposed number of experiments is the lowest required for statistically valid tests of the hypothesis. Describe below.

☐ The experiments will compare the effects of several independent variables & therefore require many groups or cohorts. Describe below.

☐ The outcome measures or phenomena being measured are variable & large sample sizes are necessary for statistically valid sampling.

☐ Differences from controls are expected to be small, & large sample sizes are necessary to distinguish differences reliably. Describe below.

☐ The experiments are technically difficult & multiple attempts will be needed to obtain satisfactory data from each experiment. Describe below.

☐ These animals will be used to produce antibodies or tissues. Describe the amount of tissue needed & how much is produced from each animal below.

☐ This is a pilot study to obtain preliminary data to determine if a larger study can be done. Describe below.

OTHER

Explain any details: Based on the tissue requirements of previous years and approximate class sizes, these numbers of animals are expected for the next 3 years.

Experimental Details

14. Breeding of animals ☒ Yes ☐ No

If yes, please describe: Adult zebrafish will be housed and cared for as described above.

15. Behavioral studies ☐ Yes ☒ No

If yes, please describe:

16. In vivo blood/fluid/tissue collection ☒ Yes ☐ No

If yes, please describe (note the tissue, collection site, frequency, volumes, and any sedation or restraint): Live mouse tissue will be obtained via cervical dislocation and decapitation as described above. Tissues collected will be used in neuroscience for physiology studies.

17. Administering any anesthetics, analgesics, therapeutic, or experimental compounds ☒ Yes ☐ No

If yes, please describe (note the compound, dose, route, and frequency): Anesthetics will include 1.3-1.5 g/kg in a ~1.5 g/5 ml urethane solution via intraperitoneal injection in mice. Urethane is used because it does not interfere with neural excitability and is only used as a terminal anesthetic to stay within AVMA 2020 Euthanasia Guidelines.

18. Surgery ☐ Yes ☒ No

If yes, please describe (note the type of surgery, expected duration, preventatives for ill effects, anesthesia, and post op care):

19. Food or Water restriction ☐ Yes ☒ No

If yes, please provide a detailed scientific justification and protocol:

20. Extended restraint ☐ Yes ☒ No

If yes, please describe (do not include short periods of restriction for blood collection or injection):

21. Introduction/Injection of biologicals (tissues, cell lines, tumors, stem cells, blood components, body fluids)

☐ Yes ☒ No

If yes, please describe:

22. Transgenic materials (will any sort of recombinant DNA materials be introduced into the animals?)

☐ Yes ☒ No

If yes, please describe:

23. Other procedures (describe any procedures not discussed above, Ex. Non-surgical procedures, monitoring, measurements, etc): Zebrafish breeding as described above.

Potential Animal Pain & Distress/Euthanasia

24. Are there any health issues due to the phenotype of the animal? ☐ Yes ☒ No

If yes, please describe the issues, how they will be recognized/monitored, and steps to alleviate the problem):

25. Describe in detail any criteria for euthanasia: If mice show any signs of pain or distress. Zebrafish demonstrate age, malformation, or wasting.

26. Method of euthanasia (The SAU IACUC requires investigators to follow the AVMA guidelines for the euthanasia of animals: 2020 edition (available online) unless justification is provided. Please include all methods of euthanasia as well as secondary physical methods used with rodents euthanized by CO2.).

Zebrafish will be euthanized via triacaine followed by decapitation as described above.

Mice will be euthanized via cervical dislocation and subsequent decapitation for fresh tissue. They will be euthanized via urethane injection and subsequent decapitation in all other cases.

Animal Locations & Transport

27. Please indicate building(s) & room number(s) of research laboratories where live animals will be transported, housed or where animal procedures will be performed.

Building	SCI	Room Number	102
Building	SCI	Room Number	203 and 203B
Building		Room Number	

28. Will the animals be transported anywhere on campus or to different buildings? ☐ Yes ☒ No

If so, please describe the transport methods:

29. If your experiment will be conducted on non SAU locations please describe in detail the facilities:

Hazardous Agents

Does this project utilize hazardous chemicals, microbial organisms, recombinant DNA, human cells, radioactive materials, or animals that carry zoonotic diseases. ☒ Yes ☐ No N

If any hazardous agents are used in live animals, please describe below. A project that uses hazardous agents may not begin until the investigator has obtained any necessary university approval. Please discuss with the IACUC to determine appropriate approval.

Agent	Urethane
BSL/ABSL level	
Specific Detail of Hazard (strain, type, etc.)	Suspected carcinogen
How is hazard used?	Terminal anesthesia

Duplicate table for each hazardous agent to be used

Are you using human embryonic stem cells?

You are responsible for knowing & following the federal, state, & SAU regulations & obtaining approval from the SAU Embryonic Stem Cell Research Oversight Committee. For more information go to the SAU Stem Cell website.

Are you using human blood or other tissue in animals?

You are responsible for knowing federal, state & SAU IRB regulations & may need to receive IRB approval prior to beginning your studies. Please read the information at the SAU IRB website.

Is any of your funding from a non-federal source (including departmental funds, gifts, private grants, clinical trial agreements, lab service agreements, & for profit contracts)? Is anyone listed on your protocol an employee, officer, or stockholder of the funding source? Do you receive income from the funding source?

You are responsible for knowing & following the SAU, state & federal regulations pertaining to Conflict of Interest. For information & application go to the Conflict of Interest website.

Will you use controlled substances as anesthetics, analgesics or test substances in this protocol?

You are responsible for knowing & following SAU rules & you must obtain a CSUA. More information & forms at EH&S Controlled Substances Acquisition, Storage, & Disposal Requirements.

Are you using hazardous agents?

You must obtain any necessary BUA, RUA, or IBC approval before starting this project. You must train your laboratory personnel & key Animal Care Program personnel regarding safe handling, disposal & clean-up of the hazardous agent. You must assure that areas where hazardous agents are used have appropriate signage & necessary PPE available.

Will you use anesthetic gases in this protocol?

Follow the Safe Use of Anesthetic Gases in Research Environments guidelines to control the risk of exposure to waste anesthetic gases.

Do you have employees or students that handle animals as a part of their job or training?

You must assure that each of your employees & students are approved for animal use and have the appropriate training, immunizations, and oversight.

Are you using animals in teaching undergraduate, graduate or continuing education students?

You must educate students on the ethical use of animals in research, appropriate handling, & student health & safety concerns.

Do you have federal grants that are supporting this protocol?

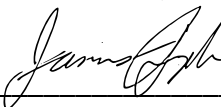
If so, NIH requires that grants receive concomitant updates with protocol amendments in some cases. NIH states: "Grantees must also obtain prior approval from NIH for changes in scope, direction, or other areas that constitute a significant change from the aims, objectives, or purposes of the approved project. The grantee must make the initial determination of the significance of the change & should consult with a Grants Management Officer as necessary."

☒ I have read all of the documents listed here that apply to my animal protocol

I am the Principal Investigator (PI) & I have thoroughly read, agree with, & will actively promote & enforce all of the assurances listed below:

1. I agree to abide by PHS Policy, USDA Regulations, SAU policies for the care & use of animals, the provisions of the ILAR Guide to the Care & Use of Laboratory Animals, & all other federal, state, & local laws & regulations governing the use of animals in research.
2. I understand that emergency veterinary care will be administered to animals showing evidence of pain or illness, in addition to routine veterinary care as prescribed for individual species. I understand that it is my responsibility to provide current & updated emergency contact information for personnel who must be contacted in an animal emergency. I understand that any unanticipated pain or distress must be reported to the veterinarian or his/her designee.
3. I assure that I have consulted a veterinarian in the preparation of this proposal, if it includes procedures that could cause pain & distress to a vertebrate animal.
4. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified biomedical scientist listed on this protocol.
5. I certify that all personnel having direct animal contact, including myself, have been trained in humane & scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, & euthanasia to be used in this project.
6. I understand that the use of hazardous agents in animals may only be initiated after approval from EH&S & I am responsible for complying with all safety related information stated under section VIII.
7. I understand that I must submit an amendment for any proposed changes to this protocol & wait for IACUC approval before beginning the work.
8. I understand that should I use the project described in this application as a basis for a proposal for funding (either extramural or intramural), it is my responsibility to ensure that the description of animal use in such funding proposals are identical in principle to that contained in this application.
9. I understand it is the responsibility of the Principal Investigator to ensure the safe & ethical conduct of all research conducted under this protocol, & to assure that all research is carried out following federal, state, local, & SAU policies governing animal research.
10. I certify that I will maintain complete, up to date & accessible records of procedures on animals as required by policy & regulation.
11. I declare that the information provided in the accompanying protocol is accurate to the best of my knowledge.
12. I certify that all state, federal & international permits for the use of the animals described in this protocol are in place (or will be in place before studies begin) including those permits mandated by the Department of Commerce, Marine Mammal Protection Act, Bureau of Land Management, National Forest Service, & foreign countries.

James Hyde
PI Full Name Printed



PI Signature

1/23/2023
Date