

# ePROTOCOL IACUC SAMPLE PROTOCOL

What follows is an example of types of answers in a "Sample Protocol". They are meant as examples and may not apply to all protocols.

IACUC - IACUC Protocol ID: IACUC-22-06-4743 (Joseph, Elaine)  
Protocol Title: Title should reflect content and subject of animal project - you can also make it similar to the grant

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**Principal Investigator**

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**Training Details**

Course ID	Course	Course Completion Date	Course Expiration Date
100	Ani Con Questionnaire	2022-04-28 00:00:00	
73559	Animal Allergy Exposure Reduction	2022-02-11 09:09:00	2025-02-10
27185	Aseptic surgery	2018-01-15 15:01:00	
27090	Biomedical Responsible Conduct of Research Course 1.	2022-02-07 10:29:00	
99756	Biosafety/Bloodborne Pathogens	2022-02-11 09:15:00	2023-02-11
203	DLAR Mouse	1900-05-01 00:00:00	
202	DLAR Rat	1900-05-01 00:00:00	
27183	Essentials for IACUC Members	2019-11-13 11:39:00	
99755	Laboratory Safety Training	2022-02-11 09:25:00	2023-02-11
132965	Radiation Awareness (Non-users)	2022-03-11 15:58:00	
27184	Reducing Pain and Distress in Laboratory Mice and Rats	2022-02-09 15:40:00	
91648	Working With Zebrafish (Danio rerio) in Research Settings	2022-02-08 14:54:00	2025-02-07
27187	Working with Mice in Research	2022-02-07 11:39:00	
27188	Working with Rats in Research Settings	2022-02-07 11:49:00	
27182	Working with the IACUC	2022-02-11 13:59:00	2025-02-10

Working with animal models? \*  Yes  No

Describe Previous Experience and Responsibilities for this Protocol: Identify the responsibilities of this individual, his/her experience with the procedures and the animal species, and who will train personnel on the procedures for work specific to this protocol.

Describe previous experience with animals and what you will be doing on this protocol. Be brief!

Is this person an emergency contact? Emergency contacts need to be able

For the Species to be Used, select each species, strain, sex, USDA Category, Source, and type in the number of animals to be used. Be sure to include any animals transferred from expiring protocols and animal animals bred in house. Categorize animals under the highest pain/distress category for all procedures they will undergo. For example, if a mouse will undergo 2 category C procedures, a category D procedure and a category E procedure, the mice should be listed as USDA category E. The total(s) in the table should match the total(s) in Question 2.

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**NUMBER OF ANIMALS** - If this is an **initial submission of a multi-year grant** beyond the three year protocol period, **all the work and number of animals** must be included in this protocol application. For all other submissions, list the total number of animals to be used over the **3 YEAR PERIOD of this protocol** (or for the life of the project if less than 3 years)

Species to be used					Add	Delete	Clone
	Species	Strain	Animal Sex	USDA Category	Source of these animals	Number	
<input type="checkbox"/>	Mouse	C57BL/6	Female	D	Purchased	1260	
<input type="checkbox"/>	Mouse	C57BL/6	Either	E	Purchased	100	

Please review the detailed [Explanation of USDA Reporting Codes](#).

**Brief examples:**

**Category B:** Animals being bred but not used for experimental purposes.

**Category C:** Experimental animals that will experience no pain or distress.

**Category D:** Experimental animals where anesthetic or analgesic agents are used to avoid pain or distress.

**Category E:** Experimental animals where anesthetic or analgesic agents cannot be used to avoid pain or distress.

1. **USDA CATEGORY E:** Identify the condition that places the animals in Category E and provide scientific justification for withholding alleviation of pain/distress. Describe any non-pharmaceutical methods that will be used to minimize pain and distress.

**NOTE:** If animals may die as a result of experimental procedures (e.g., infectious disease or oncology studies), or because an endpoint is used that allows the animals to experience significant pain or distress, justify why an alternate endpoint (e.g., weight loss, clinical signs, tumor size) cannot be used prior to death or pain or distress.

This section should contain scientific justification for why you need to withhold analgesic, or why death as an endpoint is needed, or why animals need to experience unrelieved pain and distress. Appropriate monitoring should be included, as well as citations/references where relevant.

2. **Indicate how the total number of animals needed for this study was reached for each USDA category** (group size X groups in each experiment X number of experiments). Provide the number and type of experimental and control groups in each experiment, the number of experiments planned, and the number of animals in each group. Include all animals in each USDA category, including those that will be needed for training and those that will be culled.

**The number and category of animals in this section must match the animal tables above.**

**DO NOT** cut and paste your experimental aims from your grant proposal.

Details of each procedure are to be described in the appropriate section, **NOT** here.

**B I U S x<sub>2</sub> x<sup>2</sup> I<sub>x</sub> Ω**

**A - A -**

This section should provide equations for each experiment or section of proposed project.

For example:

Experiment 1 (determine dosage):

3 strains [inserting names of strains can be helpful] X 10 animals/sample X 3 drugs [inserting names of drugs can be helpful] X 2 doses/surgery [inserting dosages can be helpful] X 2 sexes X 2 replicates = 900 (Cat D)

Experiment 2 (with one dose):

3 strains [inserting names of strains can be helpful] X 10 animals/sample X 3 drugs/surgery [inserting names of drugs can be helpful] X 2 sexes X 2 replicates = 360 (Cat D)

Experiment 3

1 strain X 10 animals/sample X 2 sexes X 5 conditions = 100 (Cat E)

General Questions – see example below for renewal protocols

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General Questions

1. Is this protocol replacing an expiring protocol?

Yes  No

The number of the expiring protocol is:

from eProtocol

a. Provide a brief summary of the work completed under the expiring protocol. It may be helpful to include the number of animals that were used/bred related to how many were approved in the expiring protocol; DLAR can provide you with a report, call 577-1107.

This should be a brief summary of what you have accomplished (it does not need to include publications), such as experiments completed and experiments in progress. You should also include the number of animals previously approved for and the number of animals used. The number of animals used can be obtained from DLAR.

b. Describe any unexpected adverse events that resulted in increased pain, distress or death rates to animals that were not described in the original protocol. Include how these were managed and what steps were taken to prevent recurrence (if applicable). Please make sure that any additional adverse effects, expected mortality, pain category changes, humane endpoints, etc. have been incorporated into this application.

You must describe any adverse events that occurred during the last three years, how they were dealt with and what you have done to prevent them from reoccurring.

c. Do you have animals currently in-house that will be transferred to this renewal protocol upon approval?

Yes  No

If yes, please include them in species section

2. Is this VA research (i.e. conducted in VA facilities, funded by the VA)?

Yes  No

Please affirm that you understand that this research cannot be initiated until after the John D. Dingell VAMC, Detroit, MI (station number 553) R&D Committee approval.

Yes  No

3. Will this protocol be submitted to the VA Central Office for approval (formerly submitted on an ACORP)?

Yes  No

4. Will this research involve students/visitors (not listed as Research Staff)?

Yes  No

If yes, the guideline will need to be affirmed  
Describe the nature of the potential participation:

5. Is this a teaching protocol?

Yes  No

How many classes are held per year?

Approximately how many students participate in each class?

6. Is this a collaboration protocol with another institution via a WSU Memorandum of Understanding?

Yes  No

Institution Name:

Select One

Institution Protocol Number:

The institution's protocol and approval letter must be attached to this application  
Describe the experimental procedures that will be conducted at WSU in this protocol application

7. Is this wildlife field research?

Yes  No

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Are You Using Section: If you are using any biological hazards (biological toxins, human cells/blood/tissues, viruses, bacteria, etc.) select use to Question 1. Add the agent below under the appropriate section.

Protocol Title: Title should reflect content and subject of animal protocol

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Are you using?

1. **BIOLOGICAL HAZARDS** - Does this research involve the use of any recombinant DNA, mammalian viruses, biological toxins, infectious agents, human blood, human cell lines, and/or human tissue?  Yes  No

a. CDC Select Agents and Biotoxins (see the list of [CDC select agents](#))  Yes  No

**Select Agents and Biotoxins**

Please click on Add to add Select Agents and Biotoxins

Note : Use of Select agents and Biotoxins requires an Institutional Biosafety Committee(IBC) Protocol application.  
IBC Protocol ID:

b. Other Toxins (non CDC select agent toxin with LD<sub>50</sub><100ng/kg)  Yes  No

**Other Toxins**

Please click on Add to add Other Toxins

Note : Use of toxins requires an Institutional Biosafety Committee(IBC) Protocol application.  
IBC Protocol ID:

c. Human or non-human primate cell lines, tumors, blood, tissues, etc.  Yes  No

**Human Cell Lines, Tumors, Blood, Tissues, etc.**

Please click on Add to add Human Cell Lines, Tumors, Blood, Tissues, etc.

d. Infectious Agents ONLY (if you will be using viral vectors to express rDNA proceed to the next question)  Yes  No

**Infectious Agents**

Please click on Add to add Infectious Agents

Note : Use of Infectious Agents requires an Institutional Biosafety Committee(IBC) Protocol application.  
IBC Protocol ID:

e. Non-plasmid rDNA, shRNA  Yes  No

Are You Using Section, cont: If you are using any chemical hazards (i.e., gas anesthesia, chemical drugs that may be hazardous to humans or animals), please list them below in Question 2. If you are not sure if they are hazardous, list them anyway. If you are using radiation hazards (i.e., PET or CT scanners, fluoroscopes, radiography, etc.), list them in Question 3.

2. **CHEMICAL HAZARDS** - Does this research involve the use of any IACUC reportable chemicals or gas anesthetic agents (e.g., isoflurane)? Please see the [WSU Reportable Chemical Guide](#) for assistance in identifying IACUC reportable chemicals.
- a. IACUC Reportable Chemicals?

**IACUC Reportable Chemical**  
Please click on Add to add IACUC Reportable Chemical

- b. Gas anesthetic agents

**Gas anesthetic agents**  
Please click on Add to add Gas anesthetic agents

- b1. Gas scavenging system?

**Gas scavenging system**  
Please click on Add to add Gas scavenging system

If No, please explain

3. **Radiation Hazard** - Does this research involve the use of non-ionizing or ionizing radiation?
- a. Non-ionizing radiation equipment-Examples include: Lasers, Infrared, Microwaves, MRI.

**Non-Ionizing radiation equipment**  
Please click on Add to add Non-Ionizing radiation equipment

- b. Ionizing radiation equipment. Examples include: PET scanner, CT scanner, SPECT, Fluoroscopy, Radiography and other imaging equipment. Please note that the use of the In Vivo Xtreme machine must be included under this section regardless of use, because it is a radiation generating machine. Contact the MICR core regarding RSC protocol.

**Ionizing radiation equipment**  
Please click on Add to add Ionizing radiation equipment

Note: Use of Ionizing Radiation Equipment requires a Radiation Safety Committee(RSC) Protocol application.

RSC Protocol ID: Select One

- c. Radioisotopes

**Radioisotopes**  
Please click on Add to add Radioisotopes

Note: Use of Radioisotopes requires a Radiation Safety Committee(RSC) protocol application.

RSC Protocol ID: Select One

Are You Using Section, cont: If you are using Physical hazards (i.e., noise, heat, cold, etc), please add it below. If you are using Controlled Substances (i.e., Ketamine, Ethiq XR, Buprenorphine, Morphine, etc.), you must have a WSU CS Protocol ID to link to your protocol. The only controlled substance DLAR will administer is Ethiq XR or buprenorphine. For non-pharmaceutical grade compounds/drugs, see [policy](#) and information below.

4. **PHYSICAL or OTHER HAZARDS** - Does this research involve the use of any physical/other hazards (e.g. nanoparticles, noise, cryogens)? Yes No

**Physical or Other Hazards**

Please click on Add to add Physical or Other Hazards

5. **CONTROLLED SUBSTANCES** - Does this research involve the use of Controlled Substances (if you are uncertain you can check the DEA CS list: [Controlled Substances - by CSA Schedule](#))? Yes No

Note : Use of Controlled Substance requires a Controlled Substance(CS) Committee Protocol application.  
CS Protocol ID: Select One v

The DLAR Staff/Veterinarians will be the ONLY individuals administering controlled substances.

6. **OCCUPATIONAL HEALTH CONCERNS** - Are there any non-routine measures, such as special vaccines or additional health screening techniques that would potentially benefit staff (e.g. research, husbandry, veterinary) that participate in or support this project? Routine measures included in the Occupational Health and Safety Program (vaccination for tetanus, rabies, and hepatitis B, and TB screening) need not be mentioned here. Yes No

Describe:

7. **Use of Non-Pharmaceutical Grade Compounds**

a. **Are any of the drugs, biologics or reagents being used in these procedures non-pharmaceutical grade (not approved for use in humans or animals)** Yes No

Identify the non-pharmaceutical grade drugs, biologics or reagents that will be administered to animals.

For this section, list any substances that will be non-pharmaceutical grade (i.e., chemical or reagent grade) - this includes substances purchased at vendors such as Sigma.

Please add justification for the use of non-pharmaceutical grade drugs (select all that apply):

No equivalent veterinary or human drug is available for experimental use.

An equivalent veterinary or human drug is available; however, the non-pharmaceutical grade is required to replicate methods from previous studies because results are directly compared to those of replicated studies.

The available human or veterinary drug is not concentrated enough to meet experimental requirements or the correct formulation for the route of administration.

The available human or veterinary drug contains preservatives or inactive ingredients confound the research goals of the study.

Scientific justification is required (scientific justification is required for use of urethane or tribromoethanol).

Other

Provide additional information to ensure proper preparation and administration of the compounds (select all that apply):

Will be filtered through sterile filter

Will use pharmaceutical grade diluents

Will use sterile diluents

Will use be stored according to manufacturer's recommendations

Other

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For the Funding Page, add all funding sources for this protocol. This includes internal and external funding sources.

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**Funding - Grants/Contracts** Add | Delete

Please click on Add to add Funding - Grants/Contracts

Funding - Other

**Dept.Funding** Add | Delete

Please click on Add to add Dept.Funding

**Other Funding (e.g. Start-up funds)** Add | Delete

Please click on Add to add Other Funding (e.g. Start-up funds)

PREVIOUS NEXT

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Purpose and Value: You can add more than one title, to coincide with grant. See instructions for lay description below


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[Purp...](#) [Anim...](#) [Anim...](#) [Proc...](#) [Non-...](#) [Surg...](#) [Sche...](#) [Euth...](#) [Atta...](#)

## PURPOSE AND POTENTIAL VALUE OF STUDY

### Official Project Title

Title should reflect content and subject of animal project - you can also make it similar to the grant

In non-technical, everyday language that a senior high school student would understand, BRIEFLY state the research or development question to be addressed in this protocol. Also, explain the potential value of this study and the ways the proposed animal use might benefit human or animal health, the advancement of knowledge, education and training, or the good of society. If there are any anticipated negative impacts (pain, distress, etc.) on the animal as a result of the proposed research, please list and explain why the data resulting from the experiments justifies this negative impact.

A scientific abstract from a grant or funding proposal is not acceptable. Do not describe experiments or procedures, or use abbreviations. The information provided in this section could be used for possible press release.

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**A-** **A-**

This should be a lay description and should avoid complicated scientific terms or define terms using lay language. It should also clearly state the objectives or goals of the research. You should state why the research is important in terms of either animal health, human health, or improving scientific knowledge. You should state why you need animals to complete the study, as opposed to cell lines or computer models. Finally, you should list any possible adverse or painful/distressful effects animals may undergo (i.e., pain from surgery, distress from food/water restriction) and weigh this against the benefits of the proposed research.

For Question 1a of the Animal Use Justification Section, enter the keywords you searched in the appropriate databases to search for alternatives to painful procedures (USDA Category D or E only). If you don't have USDA Category D/E you do not have to answer 1a.

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### Animal Use Justification

The [US Animal Welfare Act \(AWA\)](#) and [USDA Policy #12](#) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to animals (Pain and Distress Categories D and E only), and provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including **refinements, reductions, and replacements** (the 3Rs).  
**Examples of Refinement:** The use of most appropriate anesthetics and analgesics, the use of remote telemetry to increase the quality and quantity of data gathered, and humane endpoints.  
**Examples of Reduction:** The use of shared control groups, preliminary screening in non-animal systems, innovative statistical packages or a consultation with a statistician.  
**Examples of Replacement:** Alternatives such as tissue culture models, or computer-based simulations. Alternative animal models lower on the phylogenetic scale (i.e. using a mouse model in lieu of a non-human primate model).

1. **Consideration of Alternatives and the Prevention of Unnecessary Duplication.** Complete items below for Pain and Distress Categories D and E Only. Keep copies of computer database search results in your files to demonstrate your compliance with the law if regulatory authorities or the IACUC should choose to audit your project.  
 The USDA webpage [Literature Searching and Databases](#) contains links to excellent resources that can help you better understand the requirements and organize your search for alternatives.  
 WSU Medical School Contact Shiffman Medical Library via [askmed@wayne.edu](mailto:askmed@wayne.edu) or 313-577-1094  
 WSU General Libraries visit [ASK-A-LIBRARIAN](#); subject specialists are available.
  - a. **Investigators must consider less painful or less stressful alternatives to procedures, and provide assurance that proposed research does not unnecessarily duplicate previous work.** You should perform one or more database searches to meet these mandates unless compelling justifications can be made without doing so. Complete the table below for each database search you conduct to answer the questions below. The literature search must **not be older than 3 months** at time of submission of this protocol application.

Search Data		Add   Delete	
Search Date	Search Range	Keywords	Databases Searched
<input type="checkbox"/> 06/22/2022	1930-2022	enter keywords here	Medline/ Pubmed/ Web of Science

- b. **Could any of the animal procedures described in this protocol be replaced by non-animal models, such as mathematical models, computer simulations, or in vitro biological systems?** Indicate below if such replacement is or is not possible, and **provide a narrative** as on how you came to your conclusion.  
 Explain why non-animal models (computer models, cell lines, etc.) are not suitable and animals are necessary for the procedures in this protocol.
- c. **Could a smaller, less sentient mammalian species or a non-mammalian species (e.g. fish, invertebrates) substitute for the mammals in any of the experiments planned?** Indicate below if such substitution is or is not possible and **provide a narrative** on how you came to your conclusion.  
 Explain why a smaller, lesser species (i.e., rodent vs pig, fish vs rodent, invertebrate vs mammal, etc) is not appropriate for this protocol and the procedures proposed.
  - i. **Describe the biological characteristics that make each species, and sex selected the most appropriate for this project.** Cost is not an acceptable consideration.  

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x<sup>n</sup>
I<sub>x</sub>
Ω

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A-

Explain why you are using the sex(es) you chose and the species you chose - based upon their biological and phenotypical characteristics.
- d. **Could a different animal model or different animal procedure that involves (1) less distress, pain, or suffering, or (2) fewer animals substitute for any proposed animal model or animal procedure planned?** Indicate below if such replacement is or is not possible, and **provide a narrative** on how you came to your conclusion:  
 Explain if a different model or procedure that is less painful or uses fewer animals is possible. If not, please explain why.
- e. **Does the proposed research unnecessarily duplicate previous work?** If yes, provide justification for duplication.  
 State "no" if it does not or if it does, why it is necessary

Animal Use Justification Section: For the group size calculation questions, see suggestions below.

2. Indicate the METHOD(S) used to determine the group size of animals needed for this study.

**Note:** The *Guide* states that whenever possible, the number of animals requested should be justified statistically. A power analysis is strongly encouraged to justify group sizes when appropriate. Please provide this information.

- a.  Group sizes determined statistically. State what statistical analysis was performed and give the power function. The variance may be estimated from similar previously published studies. Software such as that available at [www.poweranalysis.com](http://www.poweranalysis.com) or [www.statistics.com](http://www.statistics.com) may be helpful.

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A ~~A~~

Please include the group size(s) (i.e., n=10) and details of the power analysis.

- b.  Group sizes based on quantity of harvested cells or amount of tissue required. Elaborate. (Note: A statement such as "The study requires 50 experiments" is not sufficient.)

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Please include the group size(s) (i.e., n=10) how you came up with the group size, based upon the number of cells/tissue required (i.e., 50 cells per mouse)

- c.  Pilot study or preliminary project, group variances unknown at present. **Minimal number of animals should be requested.** You must provide justification for the number of animals you are requesting. State the basis for your request.

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If this is a pilot study, please state the justification/how you came up with the sample size. It should be relatively small.

- d.  Other Elaborate and justify criteria used to determine group size.

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A ~~A~~

For Animal Breeding, Housing and Care Section, indicate the type of breeding and when weaning will occur (21 days is standard). For delayed weaning (>21 d, no more than 28 d), please list the strains which require delayed weaning. You should also indicate how you will genotype and identify pups. FYI, ear punching will accomplish both and does not require anesthesia.

Purp...
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### Animal Breeding, Housing and Care

**1. Breeding: Will animals be bred in-house?**

YES  
 NO

All animals bred in-house must be listed in the "Species" section including any excess or unsuitable animals that will not be used for experiments. For complicated breeding schemes, please consider including an attachment (see "Attachments" tab above)

a. Review the [Rodent Breeding and Weaning Policy](#) and complete the table below.

<input checked="" type="checkbox"/>	Pair mating	<input checked="" type="checkbox"/>	Pups weaned at 21 days
<input checked="" type="checkbox"/>	Trio mating	<input type="checkbox"/>	Other (describe below):
<input type="checkbox"/>	Other (describe below):		

b. Will the offspring be genotyped?  Yes  No

<input type="checkbox"/>	Tail biopsy	Review <a href="#">Rodent Tail Biopsy</a>
<input type="checkbox"/>	Toe clipping	Review <a href="#">Rodent Toe Clipping</a> (please justify below)
<input checked="" type="checkbox"/>	Other:	ear punch

**2. Rodent Identification Method (e.g. ear punch, tattoo, ear notch) See [Rodent Identification](#) for guidance.**

Not Applicable  
 None  
 List: ear punch

**3. Describe any abnormal phenotypes for strains that will be used.**

List any phenotypes that would affect behavior or physiology (i.e., health or appearance)

**4. Will Transgenic, Knockout and Knockin animals be used?**

YES (review the [Genetically-Modified Animals Guideline](#))  
 NO

a. Describe any special care or monitoring that the animals will require, or need for special breeding systems.

No special care required  
 Special care required (describe below):

b. Will these phenotypes cause an increased risk for the animal to shed intentionally introduced infectious agents, biological toxins, hazardous chemical agents, radioisotopes or create other hazards for the animal handlers and research staff?

YES Explain the hazard the animal will present to staff handling the animals and provide safety precautions required to be observed in housing and handling these animals in the space below.

NO

Animal Breeding, Housing, and Care Section: If you are housing outside of DLAR, indicate where and who is providing the care. Answer subsequent questions 4a-d. For question 7, please justify any special caging.

5. **Housing Outside DLAR Facilities: Will animals need to be maintained outside the DLAR facilities for more than 12 hours?**

YES (Review [Overnight and Long-Term Housing of Animals in Investigator Laboratories](#))

NO

>12 hours but <=24 hours

>24 hours

If animals are housed more than 24 hours in a laboratory, the room is designated as a satellite animal housing facility and must comply with all pertinent regulations as if it was a DLAR facility. If this room has not been set up as such, contact the IACUC office immediately.

**Building:**

Select One ▼

**Room:**

DLAR will provide all husbandry and oversight.\*

DLAR and PI will share the responsibilities for husbandry and oversight.\*

PI will be responsible for all husbandry and oversight. Provisions for care and housing, animal monitoring and environmental monitoring will meet or exceed standard DLAR SOPs.\*

\*All outside housing requests require a [Husbandry Agreement](#) between the Veterinarian and PI. A scanned signed agreement must be attached to this protocol, See attachments tab in the Protocol information.

In the Training Checklist section, please select the persons responsible for taking care of animals outside of DLAR facilities.

a. Which animals will be housed outside of the DLAR facilities? Please include species and specific information about which animals will be housed (e.g. post-op animals, animals undergoing behavioral testing).

b. How many animals will be housed outside the DLAR facilities at one time?

c. How long will the animals be maintained outside of the DLAR facilities?

d. Justify why it is necessary to house animals outside of the DLAR facilities?

6. **Housing Locations: Please list the buildings where animals will be housed.**

list buildings where animals will be housed

7. **Caging Requirements**

Standard housing (appropriate for species, including sterile for immunocompromised animals)

Special housing needs required (e.g. suspended wire mesh flooring, non-standard size) for **some or all** animals on this protocol. Provide justification and describe circumstances below:

If smaller than normal caging would be used (i.e., metabolic caging) or housing on wire bottom cages, justification is required and should be scientific and include duration and circumstances when animals will be in the special housing.

Animal Breeding, Housing, and Care Section: Please see below for examples and explanations for questions on special food/water, social housing, enrichment, and acclimation.

8. **Food/Water Replacement:** Please list any special food and/or water requirements (i.e., fasting, special diet, special water, etc.)

If special food/water is to be used (including periods of fasting), please list here.

9. **Social Housing:** The *Guide* states: "Single housing of social species should be the exception and justified based on experimental requirements or veterinary-related concerns about animal well-being."

Standard social housing

Single housing will be required for **some or all** animals on this protocol. Provide justification and describe circumstances below; include the duration of time animals will be singly housed:

Justification for single housing should be scientific (i.e., published references) and/or experimental (i.e., unpublished data). Describe when animals are singly housed and for how long. This includes single housing of post-operative animals.

10. **Environmental Enrichment:** The *Guide* states: "The primary aim of environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics"

Species-specific enrichment will be provided (see [Environmental Enrichment and Behavioral and Social Management of Research Animals](#) Policy/Guideline)

Enrichment will not be provided for **some or all** animals on this protocol. Provide justification and describe circumstances below:

Justification should be scientific (i.e., published references) and/or experimental (i.e., unpublished data). You should describe when enrichment won't be given and what types of enrichment. Also, add if any alternate types can be given. Also include deviation from single-housing extra enrichment requirement in this section. E.g. post-operative animals are singly-housed but cannot receive hut d/t potential to damage cranial post.

11. **State the period of time animals will be allowed to acclimate following arrival at WSU and prior to the initiation of experimental or breeding procedures** (Review the [Acclimation of Animals](#) Guideline).

Ideally, animals should be acclimated for several days before procedures begin. Only reduced acclimation periods require justification.

12. **Will photographs and/or videos of animals be taken in an animal holding facility (i.e. DLAR)?** Review the Security Policy/Guideline.

YES, list building(s) and room number(s) and describe: images to be taken and for what purpose these images will be used for (i.e., used in publication, websites, seminars, teaching) and how they'll be stored

NO

Animal Breeding, Housing, and Care Section: If animals are to be transported, answer subsequent questions as below.

If you are transporting in a hospital, answer questions 4 as “yes” and include a letter approving the transport and use of equipment in that hospital.

**13. Will animals be transported between buildings for procedures?**

- YES Review the [Transportation of Animals](#) Policy/SOP  
 NO

To ensure humane animal handling and protect against disease spread, IACUC/DLAR requires that special provisions be met regarding the transportation of animals between WSU buildings or off campus locations. **Transportation arrangements can be made through DLAR by calling 313-577-1343.**

**1. State the species and number of animals to be transported at one time:**

this should be the species and number to be transported

**2. Identify the building and room numbers involved in the transport:**

**Note:** If animals will be taken into a medical center area hospital for a procedure, you must have prior approval from the authorizing persons at that hospital.

Building and room numbers involved in the transport			Add   Delete
	FROM (WSU Building and Room)	TO (WSU Building and Room)	Round Trip
<input type="checkbox"/>	<a href="#">Biological Sciences</a>	Elliman	Y

**3. State the purpose of the transportation,** indicate if it may be necessary to do this more than once with the same or different groups of animals, and the length of the stay at each site (e.g., 1 hour, 6 hours, overnight, permanent).

State purpose of transport (i.e., imaging in Elliman) and length of stay

**4. Authorization to bring animals into WSU locations such as hospitals, clinics or access equipment in the WSU campus area used for human patients.** Provide details of authorization to use the facilities by the person responsible for the area(s). Include the name and title of the individual(s), and the date authorization was obtained. Also describe how animal use locations/equipment will be cleaned following use. An authorization letter can be attached in the "Attachments" tab above.

- NA

**5. If animals are being transported to WSU buildings, will they come into contact with, or be housed in the same room as other animals already residing at the destination?**

- Yes (provide details below):

- No

**6. Will the animals need to be sedated or anesthetized prior to or during transportation?** Note that large animals (e.g. dogs) usually require sedation prior to transportation. Please consult a veterinarian if this was not previously approved by the IACUC.

- Yes (provide details below):

- No

Animal Breeding, Housing, and Care Section: If you are performing the transportation at any time, mark “no” to question 8. FYI, DLAR will not provide transportation of hazardous animals. See below for tips on answering 8b.

**7. Will the animals be coming from a chemical or biological hazard exposure room or be shedding chemical or biological hazards?**

If yes, please see Transportation Policy for specific SOP.

**8. Will the animals be transported by DLAR?**

You are encouraged to make arrangements with DLAR to transport your animals free of charge by calling 313-577-1452 if you are considering using a personal vehicle in all circumstances.

Yes

No, provide details below. You will be required to select the person(s) responsible for transporting the animals in the Training Checklist.

**a. If you and/or your staff will be transporting the animals at any time, please assure the Committee that:**

- Animals will be transported in an appropriate climate controlled vehicle. (i.e. air conditioned/heated). The use of personal vehicles is discouraged, as it can result in allergen exposure to the occupant and future occupants of the car, as the car can serve as a potential reservoir of animal pathogens. During regular business hours, arrangements can be made with DLAR to transport animals free of charge.
- Animals will be transported expeditiously in a draped cage or cart by an approved route (out of public view avoiding personnel areas such that no one is aware that an animal is being taken into the hospital area).
- Animals will be hand carried between WSU buildings (the use of carts is discouraged due to uneven pavement conditions on walkways).
- Rodents will be transported in clean filtered microisolator cages and water bottles inverted to prevent leakage.
- Rodent cages will be sanitized at the destination. Rodent cage exteriors must be sprayed with bleach solution (1 part bleach to 20 parts water) when they reach their destination. Cages cannot be opened until the bleach solution has been on the cage for 10 minutes.
- DLAR facility leaders will be notified at least 24 hrs in advance of the return of the animals.

I will comply with the transportation requirements outlined above and have reviewed the [Transportation of Animals](#) Policy/SOP

**b. Briefly describe transportation route below and specify if this will be a vehicle or pedestrian transport.**

Transportation will be from Bio Sciences to Elliman and will occur using PIs own vehicle (Ford Edge 2022). This vehicle is regularly inspected by IACUC and follows the Transportation Policy/SOP. The use of PIs vehicle is due to unusual transportation times.

Protocol Information, Procedures Section: Add Procedures using the Add button. Make sure the Use Categories for these Procedures match the Species section.


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Animal Breeding, Hou...
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- Anim...
- Proc...
- Non-...
- Surg...
- Sche...
- Euth...
- Atta...

Use the "Add" button to select all the non-surgical procedures you plan to conduct. Once you have completed the basic information to build the table, proceed to the next tab, "Non-Surgical Procedures", to describe the details.

Procedures (Non-Surgical)

Procedures (Non-Surgical)				Add	Delete	Clone
	Procedure Type	Procedure Title	Species	Pain/Distress Category		
<input type="checkbox"/>	<a href="#">Behavioral Testing</a>	Fear Conditioning	Mouse	E		

Use the "Add" button to describe all the surgeries you plan to conduct. Each surgery must be described separately; if you will be conducting multiple surgeries you will be asked to describe how they relate to each other in the "Surgery Relationships" section.

Procedures (Surgical)

Procedures (Surgical)				Add	Delete	Clone
	Procedure Type	Procedure Title	Species	Pain/Distress Category		
<input type="checkbox"/>	<a href="#">Surgery (Mouse or Rat)</a>	Osmotic mini-pump implantation	Mouse	D		

This is an example of the “pop-up” screen where you can add a procedure. You should add all anesthetics, analgesics, and any other agents/drugs to be administered/given to animals during the procedure. Remember to hit “Save” when you are done.

**Personnel Information**

Species

General Questions

Are you using?

Funding

**Protocol Information**

Purpose and Value

Animal Use Justifica...

Animal Breeding, Hou...

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Check For Completeness

Submit Form

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Purp... Anim... Anim... **Proc...** Non... Surg... Sche... Euth... Atta...

Procedures (Non-Surgical)

**Procedure Details** Save Cancel

Procedure Type: \* Behavioral Testing

Procedure Title: \* Fear Conditioning

Species: \* Mouse Pain/Distress Category: \* E

Use Location: \* Scott Hall

Location Type: \* DLAR Facility

Room:

**Procedure Description** Save Cancel

Every procedure must be added individually and then described in the Non-Surgical Procedure Details section.

**Non-Surgical Procedure - Drugs** Save Cancel

Add any/all anesthetic agents, analgesics, drugs, etc. that will be used for this procedure.

**Anesthetic Agents** Add | Delete

Please click on 'Add' to add 'Anesthetic Agents'

**Analgesic Agents** Add | Delete

Please click on 'Add' to add 'Analgesic Agents'

**Other Agents** Add | Delete

Please click on 'Add' to add 'Other Agents'

Once you have finished adding information above, please scroll to the top and click "Save" next to Procedure Description. Do not use the "Next" button below.

This is an example of a pop-up screen where you can add a surgical procedure (a surgery is anything that requires an incision) – the example below is an osmotic mini pump implantation.

**Purp...** **Anim...** **Anim...** **Proc...** **Non...** **Surg...** **Sche...** **Euth...** **Atta...**

Personnel Information  
Species  
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**Procedures (Surgical)**

**Procedure Details** Save Cancel

Procedure Type: \*

Procedure Title: \*

Species: \*  [Pain/Distress Category:](#) \*

Use Location: \*

Location Type: \*

Room:

**Surgery Info** Save Cancel

Surgery Type: \*

Note: The USDA defines a major operative procedure as "any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions."

**Surgeon Details** Save Cancel

**Surgeon Details** Add Delete

Surgeon Name	Specific Surgical Exp.	Describe the previous experience and/or training plan to assure surgical proficiency.
<input type="checkbox"/> <a href="#">Joseph, Elaine</a>	Y	Describe previous experience

A DLAR Veterinarian will be performing or present for all surgeries.

On the same pop-up, enter the surgical details, tips for how to answer are below.

Procedure Description Save Cancel

**Surgical Procedure in Rodents**

**1. Surgical Details**  
Give a detailed overview of the surgical procedure to be performed, the size and anatomical location of incision, the anticipated time to perform the surgery, and the time frames of the performance in relation to the overall protocol. Clearly indicate the time of planned euthanasia following the surgery.

NOTE: If more than one surgery will be conducted on this protocol, you will be asked to describe how they relate to each other in a separate section.

**B I U S x<sub>2</sub> x<sup>2</sup> I<sub>x</sub> Ω**

**A ▾ A ▾**

This should be a detailed surgical description. Please include all surgical procedures, including thermal support given, supplement fluids given, etc. Specify dosages of anesthesia and analgesia given prior and during surgery. You should include animal prep (i.e., surgical site prep, instrument sterilization, etc.),

**Example**  
Surgery is performed with aseptic technique. Hair around surgical site is removed using surgical clippers. Gross debris is removed using alcohol and the surgical site is scrubbed three times alternating with chlorhexidine (or providone-iodine) followed by sterile water (or alcohol). Ophthalmic ointment is applied to eyes. Sterile instruments will be used, along with sterile surgical drapes. Proper PPE will be used including sterile surgical gloves, surgical caps, surgical masks, and sterile gowns.

Animals will be given Ethiq<sup>a</sup> XR (3.25 mg/kg, SQ) and then anesthetized with isoflurane (5% initially, reduced to 2% for the duration of the procedure) with a nose cone and placed in dorsal recumbency. Depth of anesthesia will be checked by toe pinch. The skin and instruments are prepared as described above. Anesthetic depth is assured and monitored through toe pinch. After adequate anesthesia and buprenorphine administration, a ventral incision will be made in order to separate skin, muscle, and fascia and gain access to the peritoneal cavity. Through the opening, a sterile osmotic minipump will be placed in the peritoneal cavity. The body wall incision will be closed by suturing (absorbable PDS sutures), then the skin incision closed using wound clips or sutures. After 7-14 days, surgical wound clips or suture will be removed using sterile wound clip forceps or scissors.

Pre/Intra operative details Save Cancel

**2. List pre/intra-operative analgesia, anesthesia, sedation, and muscle relaxation as well as any pre-treatment:**

**Anesthetic Agents** Add Delete

Agent Name	Dosage (in mg/kg if possible)	Route
<input type="checkbox"/> Isoflurane	2-5%	Inhalation (INH)

Finally, indicate the anesthetics, analgesics, or other agents and how you will monitor the animals post-operatively.

Anesthetic Agents			Add   Delete
Agent Name	Dosage (in mg/kg if possible)	Route	
<input type="checkbox"/> Isoflurane	2-5%	Inhalation (INH)	

Indicate what parameters will be used to determine the need for additional doses of anesthesia.

Toe pinch, whisker twitch

Analgesic Agents			Add   Delete
Agent Name	Dosage (in mg/kg if possible)	Route	
<input type="checkbox"/> Ethiqo XR	3.25 mg/kg	Subcutaneous (SQ)	

Other Agents	Add   Delete
Please click on 'Add' to add 'Other Agents'	

Post operative details	Save   Cancel
3. Will post-operative analgesia or other treatment be administered?	<input type="radio"/> Yes <input type="radio"/> No

Analgesic Agents
Please click on 'Add' to add 'Analgesic Agents'

Other Agents
Please click on 'Add' to add 'Other Agents'

4. How will the animals be monitored for adverse effects? Describe any potential effects.

Example:  
Animals will be monitored for signs of post-surgical pain and infection or other complications. Boost and food pellets will be placed on the floor of the cage for easy access. In addition, mice will be monitored a minimum of once a day for the first 7 days following the surgery, and 2x weekly for the duration of the study. To determine whether the animals have pain, distress, and infection, the PI or study personnel will specifically pay attention to the following parameters: For infection, drinking, eating, and walking patterns will be monitored. For pain, the presence of awkward gait, hunched back, ocular and/or nasal discharge, and aggressive behavior will be monitored. If any animal manifests signs of pain or infection, the veterinarian will be consulted and appropriate treatment provided.

Non-Surgical Procedure Details: This should be a summary/narrative of all procedures that are proposed in the protocol. You should make sure to list/describe all procedures in Procedures list.

#### Non-Surgical Procedure Details

##### Procedures

Species	Procedure Title	Procedure Type	Pain Category
Mouse	Fear Conditioning	Behavioral Testing	E

**DESCRIBE ALL NON-SURGICAL PROCEDURES:** Summarize in a narrative what procedures will be done. Include only those experiments where animals are directly involved. When animals are used as donors of organs, tissues, or cells, only describe how the organs, tissues or cells will be obtained. Do not describe what will be done with those organs, tissues or cells once they have been removed from the animal.

1. Describe every procedure.



The protocol you submit is a "stand alone" document. Do not refer to procedures in other protocols or publications or assume that they are so generally understood or used that everyone will know what you will do. This section should correlate with what you included in the Experimental Timeline and should include a brief experimental design statements for each experiment. The committee does not require descriptions of in vitro experiments.

This section is for the reviewers. It is important that they understand the experimental plan in detail, so that they know what happens to each animal throughout the experiment, from beginning to end, and also that they understand how the experimental design accomplishes the goals of the research. Please use consistent names/labels that correspond with the procedure titles. If a procedure or drug has multiple names, use one and stick with it. Don't vary it throughout the text.

For breeding protocols, make sure you explain the breeding system (monogamous, trio breeding, etc.) as well as when and how you will wean the animals, genotype the animals, and tag or identify the animals.

For experimental protocols an example follows:

In this study, we propose to conduct an experiment to determine whether social stress will produce an escalation on ethanol consumption. We hypothesize that greater effects will be seen in FVB/NJ mice as we have seen for the intermittent access procedure.

Experiment 1: Social Defeat induced escalation of .....

Methods and Design:

[list all methods here including amounts of compounds given/injected/etc., amounts of blood/urine/etc taken and describe the overall experimental design, for example]

A. Fear Conditioning Behavior Tesing ....

2. How will the animals be monitored for adverse effects? Describe any likely effects.

Describe how often animals will be monitored (every other day, once a week) and what they will be monitored for (poor body condition (body condition score <2); weight loss > 20%; labored breathing; signs of severe illness including scruffy, hunched and inactive). More invasive procedures, or those that have the potential to induce more or more severe adverse effects may require more intensive monitoring. Monitoring should be specifically described for those circumstances.

If weight loss of 20% is selected as an endpoint, regular monitoring of weight should be performed and described here. If weight will not be monitored, body condition scoring (BCS < 2/5) should be used as an endpoint.

Surgery Relationships: If more than one surgery has been proposed, indicate if multiple survival surgeries will occur on the same animal at any time in the protocol. If yes, you must provide justification (question 1a) and explain the order of the surgeries and time between them (question 2).

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- Surgery Relationships**
- Schedule of Procedures
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- Anim...
- Anim...
- Proc...
- Non-...
- Surg...**
- Sche...
- Euth...
- Atta...

Surgical Procedures			
Species	Procedure Title	Procedure Type	Pain Category
Mouse	Osmotic mini-pump implantation	Surgery (Mouse or Rat)	D

1. Will this project include multiple survival surgeries on the same animal?

**1a. Multiple survival** surgeries will be conducted on an animal, review the Multiple Survival Surgeries Policy and provide justification below.

2. Describe the sequence and timing of the surgeries and how they relate to each other. If multiple surgeries will be conducted on some or all of the animals use enough details to allow the reviewers to understand what each animal will undergo.

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A ▾ A ▾

Schedule of Procedures section (see explanation below)

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- Anim...
- Proc...
- Non-...
- Surg...
- Sche...**
- Euth...
- Atta...

Schedule of Procedures			
Species	Procedure Title	Procedure Type	Pain Category
Mouse	Fear Conditioning	Behavioral Testing	E
Mouse	Osmotic mini-pump implantation	Surgery (Mouse or Rat)	D

Schedule of procedures for experimental groups: State or list in chronological order all procedures for each experimental group, their frequency, and time points over the course of the experiment. Details of each procedure are to be described in the appropriate sections, NOT here. A diagram or chart may be helpful to explain complex designs, which can be added in the "Attachments" tab above.

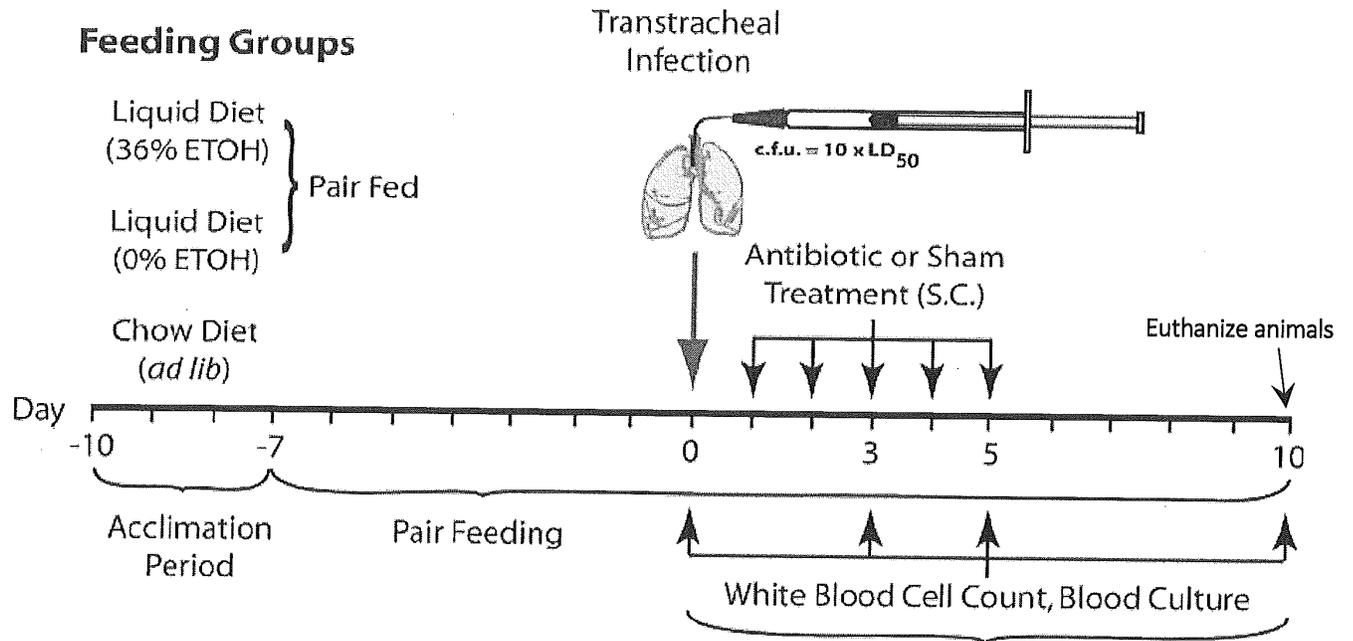
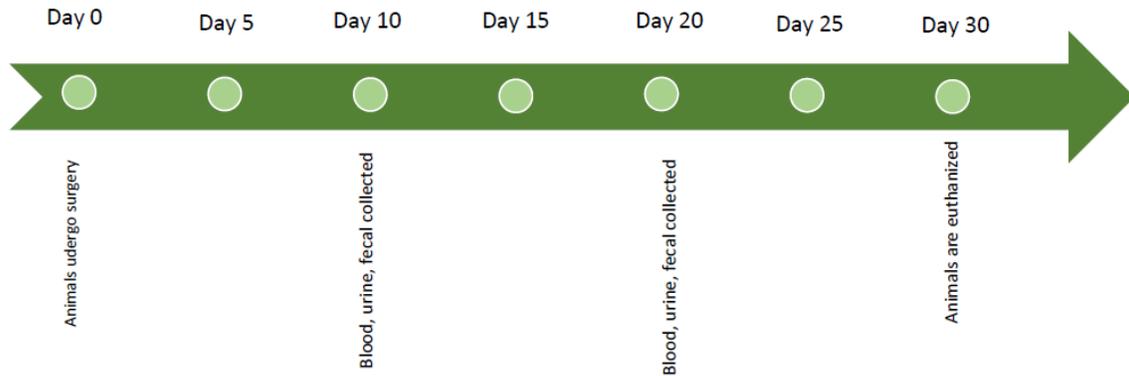
**B** *I* U ~~S~~  $x_2$   $x^2$   $I_x$   $\Omega$

**A** **A**

This section should be a clear timeline of ALL procedures from start of experiment to euthanasia and should match the Species question 2. See examples of flowcharts below or this example:  
Day 1: Surgery.  
Day 8-20: Behavioral Testing 2 times weekly  
  
Day 30: Euthanize

Example Timelines that could be uploaded as Attachments

## Timeline



Euthanasia section: State conditions that would warrant euthanasia (i.e., humane endpoints) as well as the methods of euthanasia for each species.

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1. **STATE the SPECIFIC CRITERIA for the euthanasia of abnormal or moribund animals (assume someone may have to euthanize animals IN YOUR ABSENCE). Review the [Defining Humane Endpoints and End-stage Illness](#) Guideline.**

**Not Applicable (e.g. animals are used for tissue harvesting only and will not undergo any procedures prior to death)**

Weight loss of 20% or more

Other conditions (examples may include, but are not limited to: a clinical condition that does not respond to treatment, such as an infected surgical site; any condition that a veterinarian deems severe enough to warrant euthanizing the animal). **Please describe below:**

This should include examples such as severe infection of surgical site, signs of severe illness including scruffy, hunched and inactive; self-mutilation, etc.

PHS Policy on Humane Care and Use of Laboratory Animals requires the IACUC to use the recommendations of the [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](#); please refer to it when necessary. If anesthetic overdose or CO2 narcosis is used, a secondary procedure such as bilateral pneumothorax, severing the aorta, or removal of a critical organ must be used to assure that the animal will not recover.

Euthanasia					Add   Delete
Species	Primary euthanasia method	Route of Administration	Dosage mg/kg (if possible)	Secondary euthanasia method	
<input type="checkbox"/> <a href="#">Mouse</a>	Carbon dioxide	Inhalation (INH)	30-70%	Cervical Dislocation	
<input type="checkbox"/> <a href="#">Mouse</a>	Cervical dislocation			N/A	

2. **Are your methods of euthanasia consistent with the [WSU Methods of Euthanasia Policy](#) such that they do NOT require scientific justification?**

If no, please provide scientific justification for the use of the method below.

Yes  No

3. **Who will be responsible for performing euthanasia?**  
Personnel Details

Personnel Responsible		Add   Delete
Name	Experience: Yes or No	
<input type="checkbox"/> <a href="#">Joseph Elaine</a>	Y	

The DLAR Staff/Veterinarians will be the ONLY individuals performing euthanasia.

4. **Does this research include the euthanasia of mouse and/or rat fetuses and neonates?**

Yes  No

Review and affirm the [Euthanasia of Mouse and Rat Fetuses and Neonates](#) in the Guidelines section. If it will not be followed then the variance must be justified in that section.

5. **Will all animals be euthanized at the end of this study?**

Yes  No

If no, state their final disposition:

If animals are to be adopted after study or donated, describe here.

For euthanasia that requires justification, see example below.

**Note: \* denotes mandatory field.**

<b>Euthanasia</b>		Save   Cancel
<b>Species *</b>	Mouse	▼
<b>Primary Method of Euthanasia *</b>	Cervical dislocation	▼
<b>Will the animal be anesthetized or sedated? *</b>	<input type="button" value="Yes"/> <input checked="" type="button" value="No"/>	
<b>Justification for not using sedation *</b>	<input type="text" value="This justification should be experiment specific and scientific"/>	
<b>Secondary Method of Euthanasia</b>	N/A	▼

Finally, if training is complete, you will perform a Certification, COI Disclosure, and submit to your Department Chair for Approval.