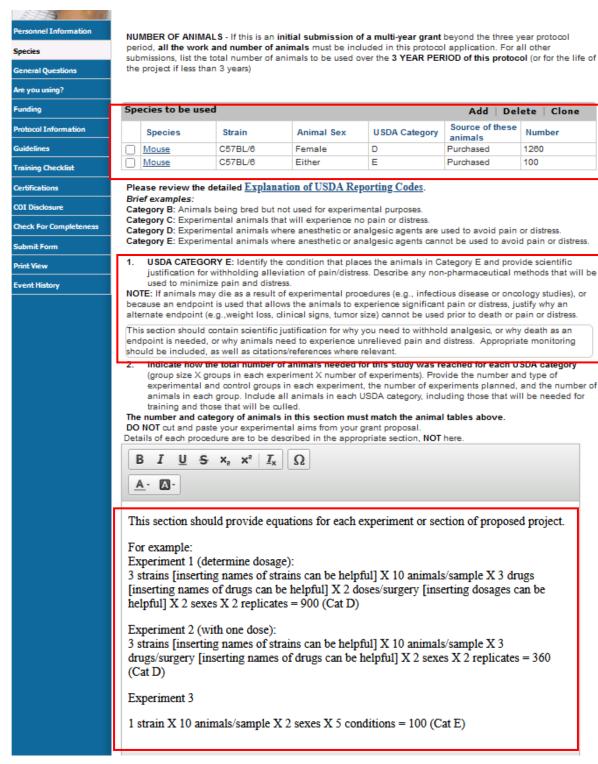
## **ePROTOCOL IACUC SAMPLE PROTOCOL**

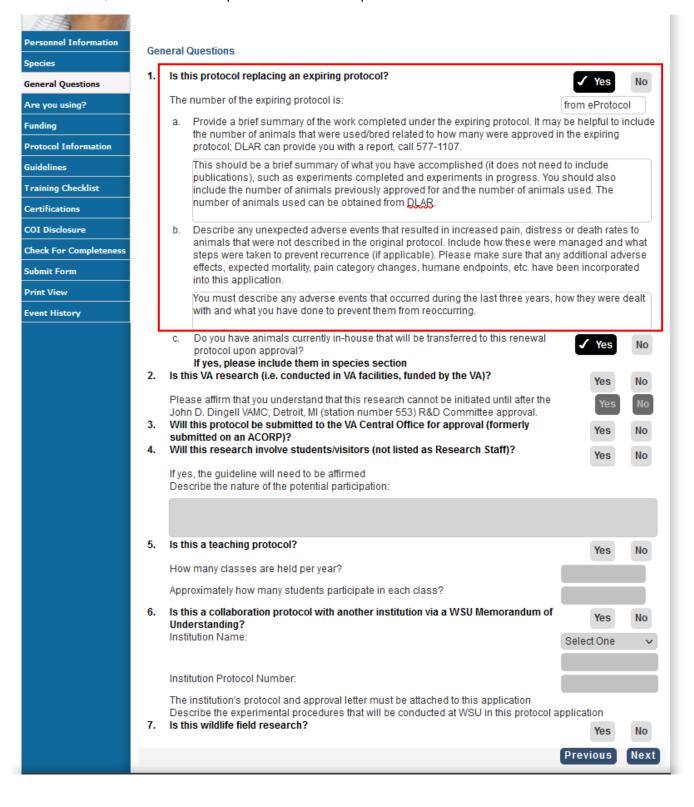
What follows is an example of types of answers in a "Sample Protocol". They are meant as examples and may not apply to all protocols.

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7	3559	Animal Allergy Exposur	e Reducti	on	2022-02-11 09:09:00	2025-02-10
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9	9756	Biosafety/Bloodborne P	athogens		2022-02-11 09:15:00	2023-02-11
2	03	DLAR Mouse			1900-05-01 00:00:00	
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2	7183	Essentials for IACUC Me			2019-11-13	2023-02-11
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	1648	Settings Working with Mice in Re		, III NESEAIGI	14:54:00 2022-02-07	2020-02-01
	7187	Working with Rats in Re		ettings	11:39:00 2022-02-07	
	7188	Working with the IACUC			11:49:00 2022-02-11	2025-02-10
2	7182				13:59:00	
V	Vorking with a	nimal models? *				✓ Yes No
t	his individual,	ous Experience and Res his/her experience with the procedures for work s	the proc	edures and the ani		
Ē	Describe previou	us experience with anima	Is and wh	at you will be doing	on this protoco	I. Be brief!

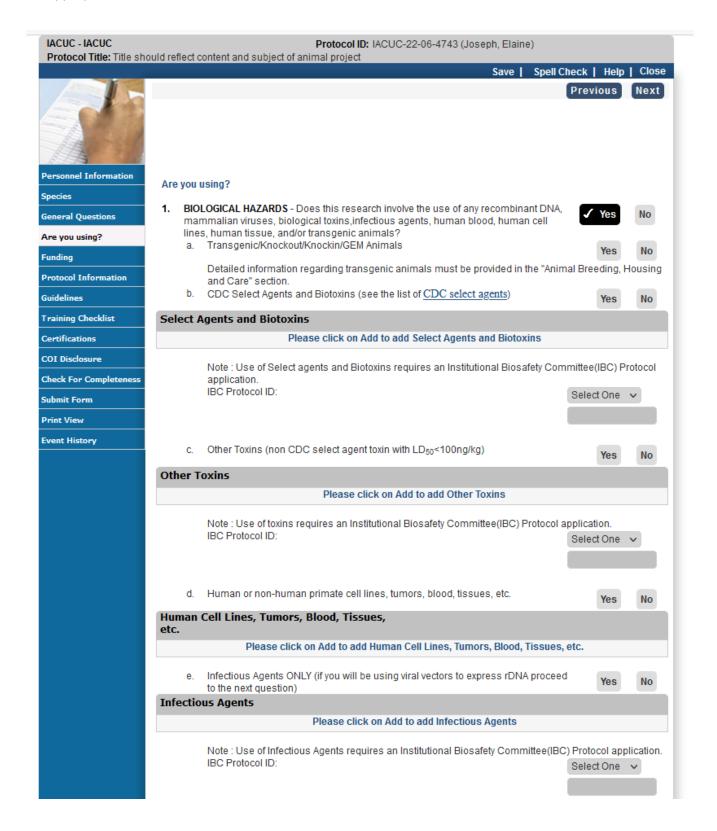
For the Species to be Used, select each species, strain, sex, USDA Category, Source, and type in the number of animals to be used. Be sure to include any animals transferred from expiring protocols and animal animals bred in house. Categorize animals under the highest pain/distress category for all procedures 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.



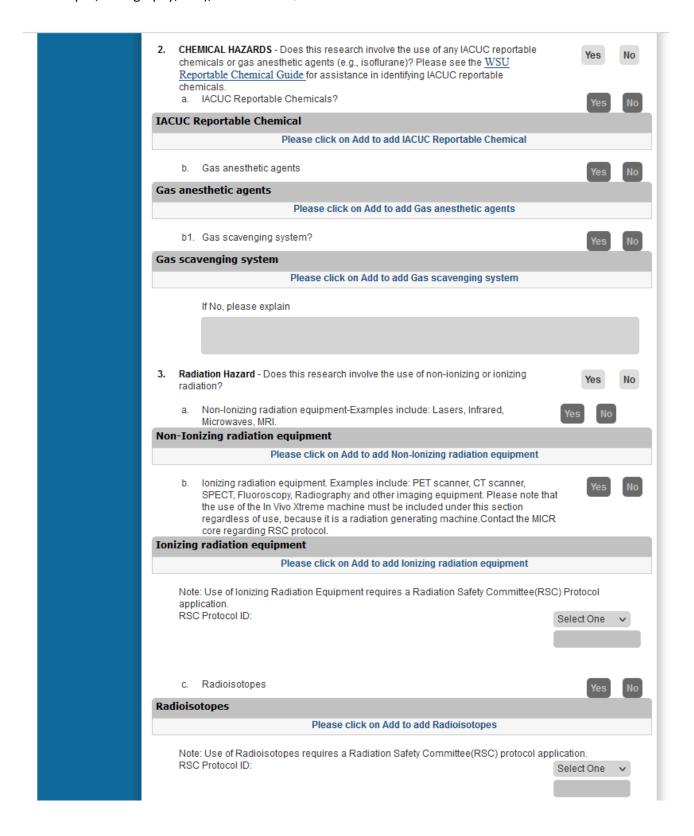
## General Questions – see example below for renewal protocols



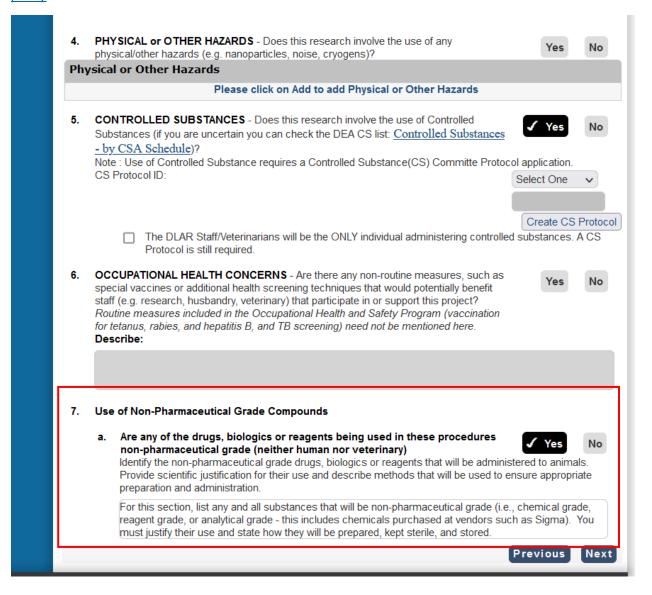
Are You Using Section: If you are using any biological hazards (biological toxins, human cells/blood/tissues, viruses, bacteria, etc.) select use to Question 1. Add the agent below under the appropriate section.



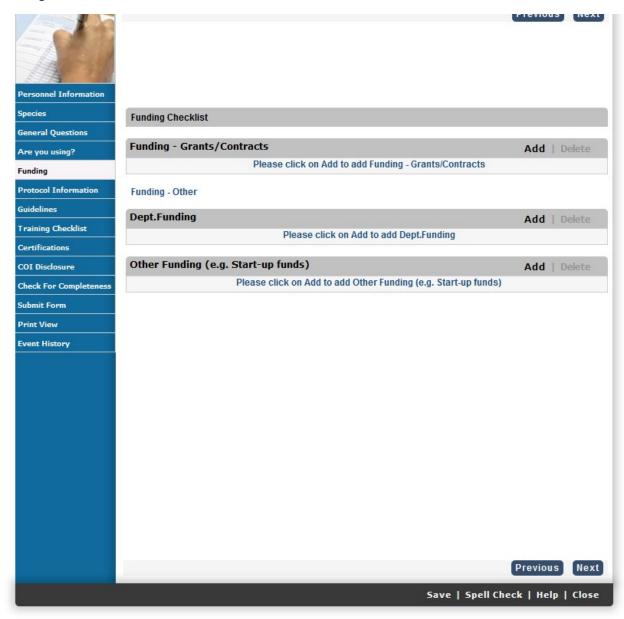
Are You Using Section, cont: If you are using any chemical hazards (i.e., gas anesthesia, chemical drugs that may be hazardous to humans or animals), please list them below in Question 2. If you are not sure if they are hazardous, list them anyway. If you are using radiation hazards (i.e., PET or CT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



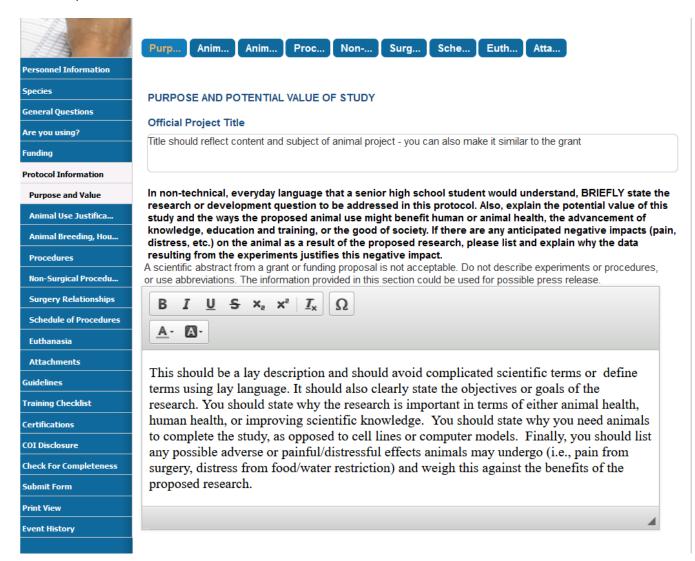
Are You Using Section, cont: If you are using Physical hazards (i.e., noise, heat, cold, etc), please add it below. If you are using Controlled Substances (i.e., Ketamine, Ethiqa XR, Buprenorphine, Morphine, etc.), you must have a WSU CS Protocol ID to link to your protocol. The only controlled substance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



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



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



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.



#### Animal Use Justification

The <u>US Animal Welfare Act</u> (AWA) and <u>USDA Policy #12</u> 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).

 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 <u>Literature Searching and Databases</u> 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 <u>askmed@wayne.edu</u> or 313-577-1094

WSU General Libraries visit <u>ASK-A-LIBRARIAN</u>; 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
	08/22/2022	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.

 Describe the biological characteristics that make each species, and sex selected the most appropriate for this project. Cost is not an acceptable consideration.



Explain why you are using the sex(es) you chose and the species you chose - based upon their biological and phenotypical characteristics.

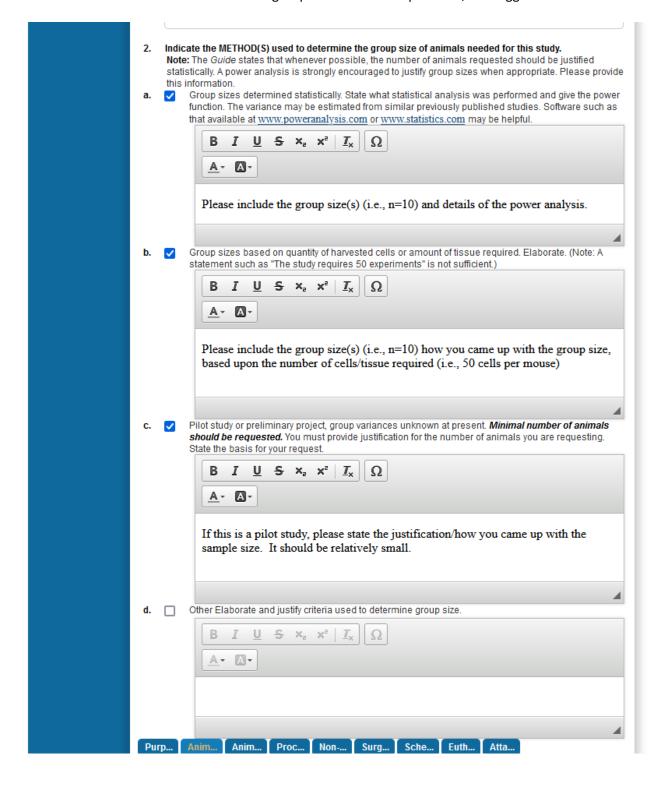
d. Could a different animal model or different animal procedure that involves (1) less distress, pain, or suffering, or (2) fewer animals substitute for any proposed animal model or animal procedure planned? Indicate below if such replacement is or is not possible, and provide a narrative on how you 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.

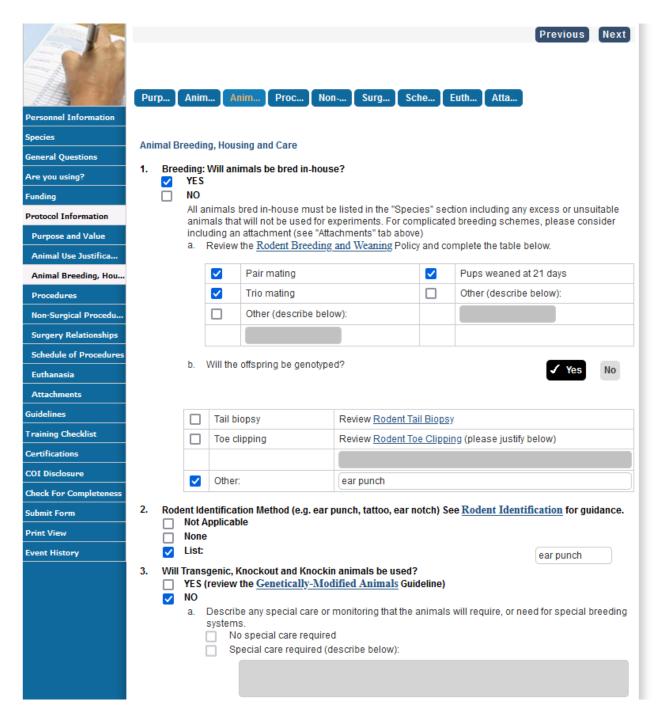
 Does the proposed research unnecessarily duplicate previous work? If yes, provide justification for duplication.

State "no" if if does not or if it does, why it is necessary

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



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.



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 5, please justify any special caging.

than 1	<ul> <li>&gt;12 hours but &lt;=24 hours</li> <li>&gt;24 hours</li> <li>If animals are housed more than 24 hours in a laboratory, the room is designated a housingfacility and must comply with all pertinent regulations as if it was a DLAR fainant not been set up as such, contact the IACUC office immediately.</li> </ul>		
E	duilding:	Select One 🗸	
F	doom:		
	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 monitoring and environmental monitoring will meet or exceed standard DLAF.  *All outside housing requests require a <a href="Husbandry Agreement">Husbandry Agreement</a> between the scanned signed agreement must be attached to this protocol, See attachment information.  In the Training Checklist section, please select the persons reponsible for ta outside of DLAR facilities.	R SOPs.*  DLAR and Pl. A  nts tab in the Protocol	
	a. Which animals will be housed outside of the DLAR facilities? Please include information about which animals will be housed (e.g. post-op animals, anim behavioral testing).		
	b. How many animals will be housed outside the DLAR facilities at one time?		
	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?		
□ S ✓ S	g Requirements Itandard housing (appropriate for species, including sterile for immunocomprom Itandard housing needs required (e.g. suspended wire mesh flooring, non-standar It animals on this protocol. Provide justification and describe circumstances below If unclude smaller than normal caging (i.e., metabolic caging) and housing or Itandard ages. Justification should be scientific and include duration and circumstances are in the special housing.	rd size) for <b>some or</b> w: n wire bottom	

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

ju D	stified based on experimental requirements or veterinary-related concerns about animal well-being.'  Standard social housing
C	Single housing will be required for <b>some or all</b> 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.
er ar w	nvironmental Enrichment: The <i>Guid</i> e states: "The primary aim of environmental enrichment is to nhance animal well-being by providing animals with sensory and motor stimulation, through structure of resources that facilitate the expression of species-typical behaviors and promote psychological ell-being through physical exercise, manipulative activities, and cognitive challenges according to becies-specific characteristics"
Ċ	Species-specific enrichment will be provided (see Environmental Enrichment and Behavioral and
	Social Management of Research Animals Policy/Guideline)
V	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.
	tate the period of time animals will be allowed to acclimate following arrival at WSU and prior to the itiation of experimental or breeding procedures (Review the <u>Acclimation of Animals</u> Guideline).
Id	eally, animals should be acclimated for several days before procedures begin. Only reduced acclimation eriods require justification.
jo po po po po po po po po po po po po po	eriods require justification.
jo D. W	ill photographs and/or videos of animals be taken in an animal holding facility (i.e. DLAR)? Review theourity 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
ld pe	ill photographs and/or videos of animals be taken in an animal holding facility (i.e. DLAR)? Review the curity 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 theyll be stored:

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.

YES Review the <u>Transportation</u> NO ensure humane animal handling the transports of t	on of Animals Policy/SOP ng and protect against disease spr ansportation of animals between V	VSU buildings or off campus			
State the species and number of animals to be transported at one time:					
This should be the species (i	.e., mouse) and number to be trans	sported.			
te: If animals will be taken into	a medical center area hospital for a		rior		
_	ved in the	Add   [	Delete		
M (WSU Building and Room)	TO (WSU Building and Room)	Round Trip			
ogical Sciences	Elliman	Υ			
the same or different groups overnight, permanent).  State purpose of transport (i.e.  Authorization to bring anima the WSU campus area used the person responsible for th authorization was obtained. A	of animals, and the length of the state, for imaging in Elliman) and length of the state, for imaging in Elliman) and length of the state of the sta	ay at each site (e.g., 1 hour, 6 th of stay at site. spitals, clinics or access equals of authorization to use the fee of the individual(s), and the clean	uipment in acilities by		
the same room as other ani	mals already residing at the destir		housed in		
✓ No					
		r during transportation? Not on. Please consult a veterina			
	YES Review the Transportation NO  ensure humane animal handling ovisions be met regarding the transportation arrangements can be stated the species and numb. This should be the species (in the species of the species of the transportation arrangements can be stated the species of the species of the species of the species of the transport of the same or different groups overnight, permanent). State purpose of transport (i.e. the same or different groups overnight, permanent).  Authorization to bring animate the WSU campus area used the person responsible for the authorization was obtained. A following use. An authorization was obtained. A following use. An authorization was obtained. Yes (provide details below the same room as other animals are being transport of the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals are details below the same room as other animals.	ensure humane animal handling and protect against disease sprovisions be met regarding the transportation of animals between Vansportation arrangements can be made through DLAR by callin  State the species and number of animals to be transported at This should be the species (i.e., mouse) and number to be transported at This should be the species (i.e., mouse) and number to be transporte: If animals will be taken into a medical center area hospital for a proval from the authorizing persons at that hospital.  If animals will be taken into a medical center area hospital for a proval from the authorizing persons at that hospital.  If animals and Room and the length of the state overnight, permanent.  State the purpose of the transportation, indicate if it may be nearly the same or different groups of animals, and the length of the state overnight, permanent).  State purpose of transport (i.e., for imaging in Elliman) and length the state overnight, permanent).  Authorization to bring animals into WSU locations such as hose the WSU campus area used for human patients. Provide detail the person responsible for the area(s). Include the name and titl authorization was obtained. Also describe how animal use located following use. An authorization letter can be attached in the "Attacolor NA.  If animals are being transported to WSU buildings, will they contain the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already residing at the destination of the same room as other animals already res	YES Review the Transportation of Animals Policy/SOP NO  ensure humane animal handling and protect against disease spread, IACUC/DLAR requires to towisions be met regarding the transportation of animals between WSU buildings or off campus ansportation arrangements can be made through DLAR by calling 313-577-1343.  State the species and number of animals to be transported at one time:  This should be the species (i.e., mouse) and number to be transported.  Identify the building and room numbers involved in the transport:  tet: if animals will be taken into a medical center area hospital for a procedure, you must have proval from the authorizing persons at that hospital.  g and room numbers involved in the ort.  M (WSU Building and Room)  TO (WSU Building and Room)  Round Trip  Guical Sciences  Elliman  Y  State the purpose of the transportation, indicate if it may be necessary to do this more than the same or different groups of animals, and the length of the stay at each site (e.g., 1 hour, 6 overnight, permanent).  State purpose of transport (i.e., for imaging in Elliman) and length of stay at site.  Authorization to bring animals into WSU locations such as hospitals, clinics or access equive WSU campus area used for human patients. Provide details of authorization to use the function was obtained. Also describe how animal use locations/equipment will be clean following use. An authorization letter can be attached in the "Attachments" tab above.  NA  If animals are being transported to WSU buildings, will they come into contact with, or be the same room as other animals already residing at the destination?  Yes (provide details below):		

Animal Breeding, Housing, and Care Section: If you are performing the transportation at any time, mark "no" to question 7. FYI, DLAR will not provide transportation of hazardous animals. See below for tips on answering 7b.

## 7. 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 Biological Sciences to Elliman and will occur using the Pl's own vehicle (Ford Edge 2022). This vehicle will be regularly inspected by IACUC and following the

Purp...

Anim...

Proc...

Non-...

Transportation Policy/SOP. Use of Pt's vehicle is due to unusual transportation times.

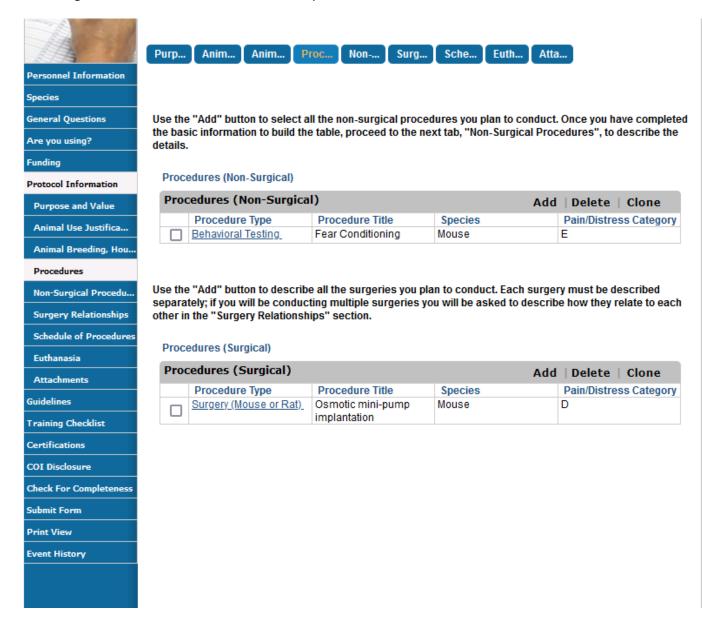
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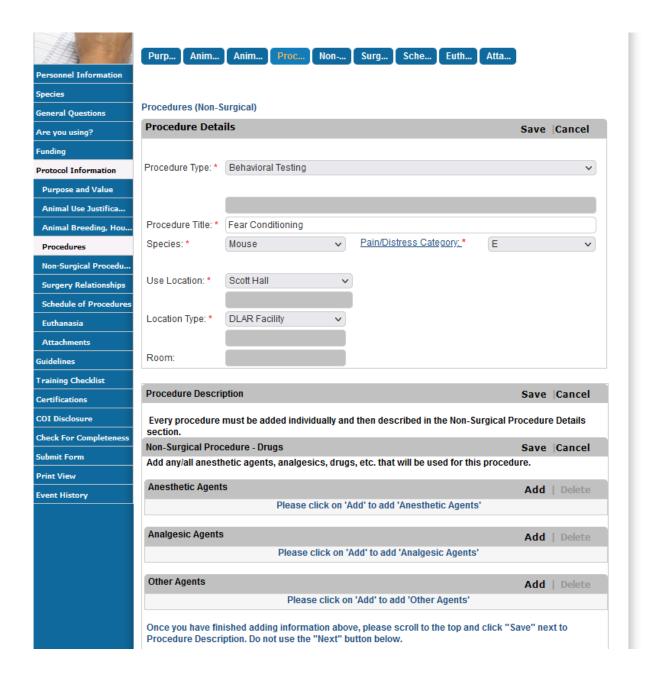
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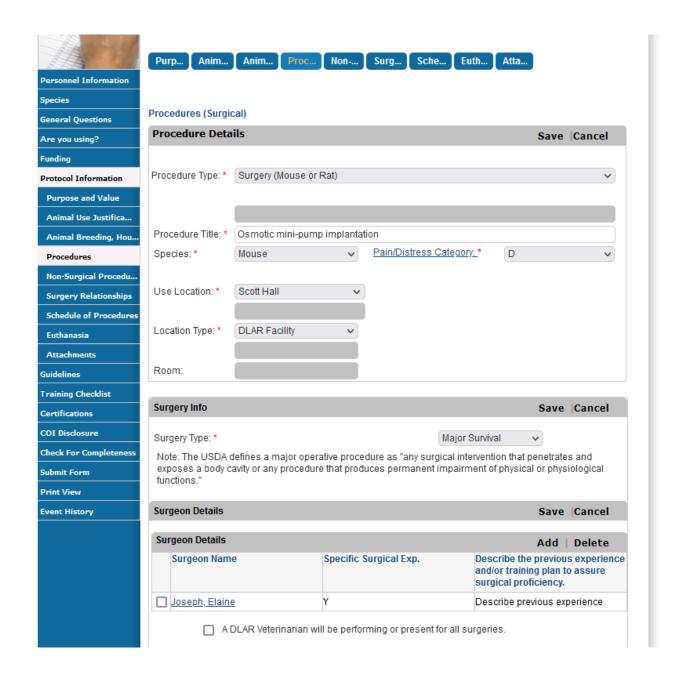
Protocol Information, Procedures Section: Add Procedures using the Add button. Make sure the Use Categories for these Procedures match the Species section.



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.



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.



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.



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 Ethiqa 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.

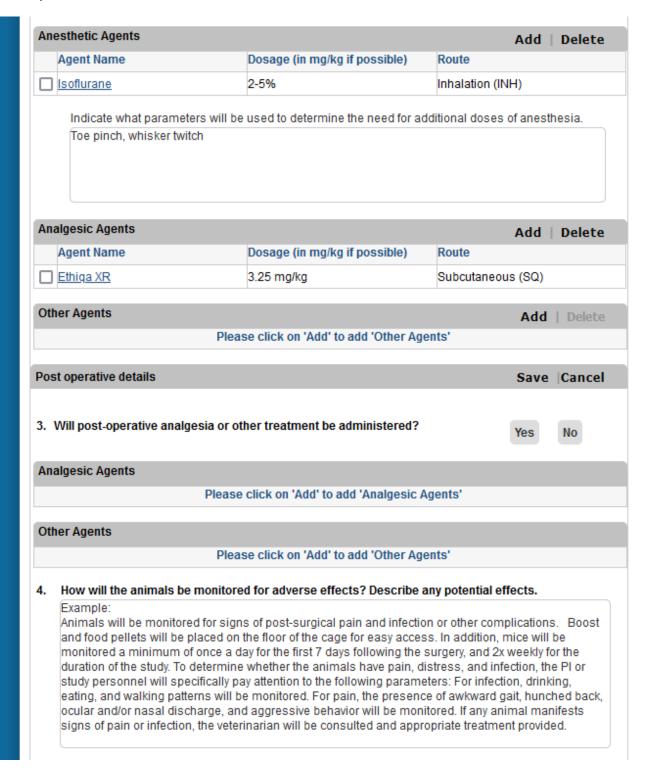
## Pre/Intra operative details

Save | Cancel

List pre/intra-operative analgesia, anesthesia, sedation, and muscle relaxation as well as any pretreatment:

Anesthetic Agents Add   Dele			
	Agent Name	Dosage (in mg/kg if possible)	Route
	<u>Isoflurane</u>	2-5%	Inhalation (INH)

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



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.

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]

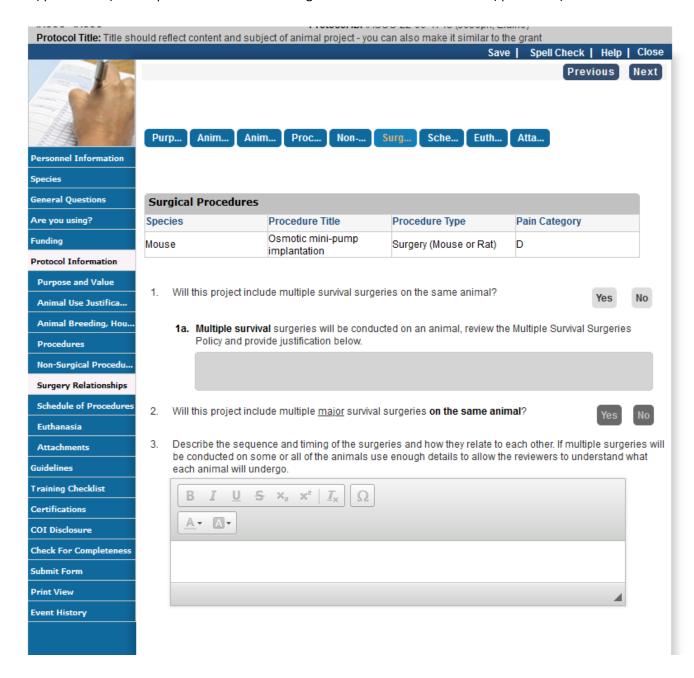
A. Fear Conditioning Behavior Tesing ....

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.

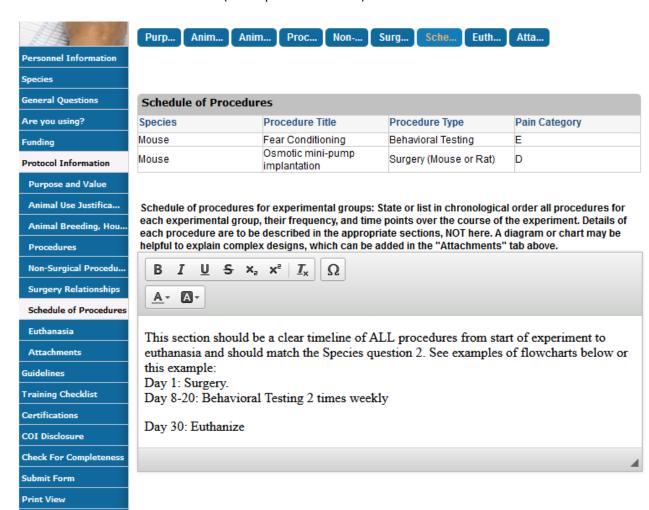
If weight loss of 20% is selected as an endpoint, regular monitoring of weight should be performed and described here. If weight will not be monitored, body condition scoring (BCS < 2/5) should be used as an endpoint.

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 3).

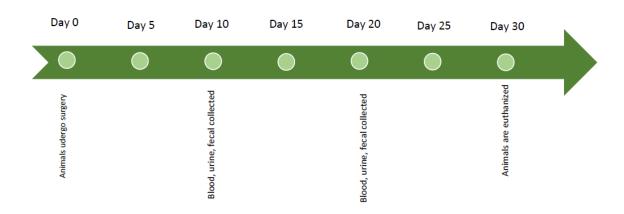


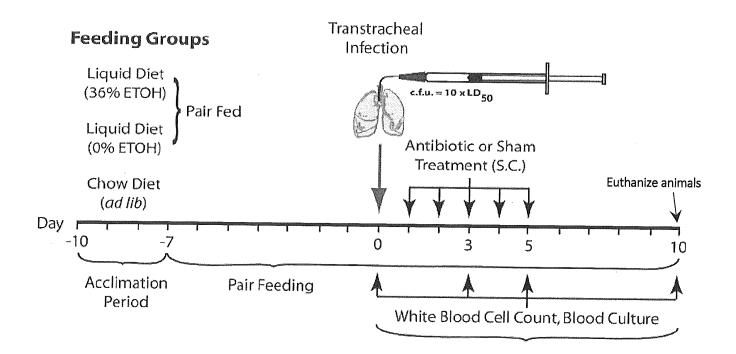
## Schedule of Procedures section (see explanation below)

**Event History** 

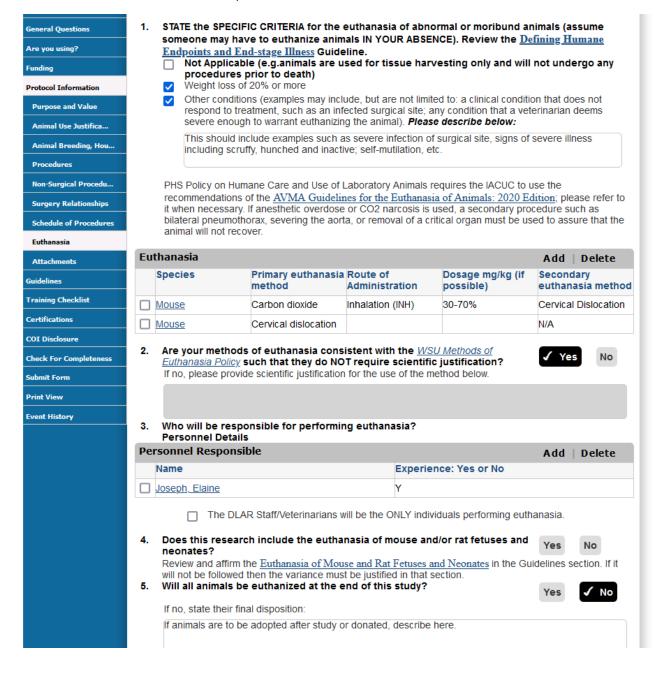


# **Timeline**

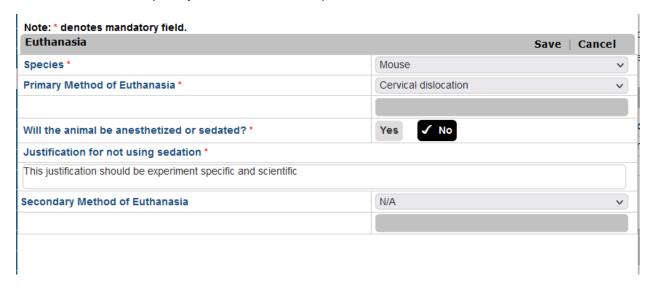




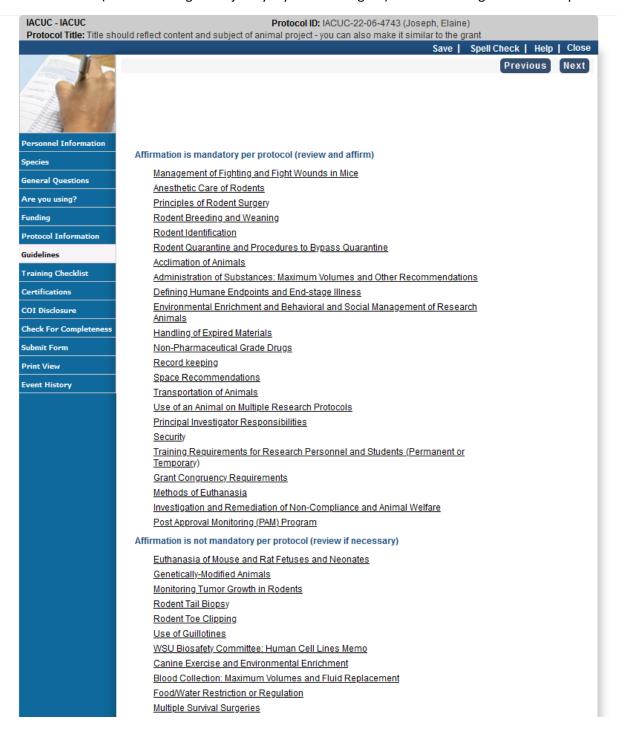
Euthanasia section: State conditions that would warrant euthanasia (i.e., humane endpoints) as well as the methods of euthanasia for each species.



For euthanasia that requires justification, see example below.



You must review (click on and agree or justify if you do not agree) to each of the guidelines and policies.



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