University of Wisconsin-Madison
Institutional Animal Care and Use Committee (IACUC)
IACUC Protocol Application

Protocol # : V006315
Date Approved : 6/2/2021
Expiration date : 6/1/2024

Protocol Basics

Terminated 7/19/2022

1. Protocol Title

* Give your protocol a title.

> Disabled submarine scenario to determine the feasibility of the current US Submarine Rescue System Decompression Plan (SRSDP) using a sheep model

2. Principal Investigator (PI)

If you cannot find the name you want, email arrow help@rarc.wisc.edu.

* Select the Principal Investigator (PI).

[redacted]

3. PI Status

* Select the current status of the listed PI.

- (•) **Faculty**
- ( ) Emeritus appointment
- ( ) Other

4. PI Department

* Enter the PI's department name.

> [redacted]

5. Protocol Renewal

* Is this application a renewal of a previously approved paper protocol?

○ Yes ◉ **No**

6. Protocol Writers

If you cannot find a name or have other questions, email arrow help@rarc.wisc.edu

Other than the PI, choose people to help prepare, edit and submit protocols.

Person
There are no items to display

7. Email Contacts

If you cannot find the name you want, email arrow help@rarc.wisc.edu

Along with the PI and protocol writers, add up to two people who should receive pertinent protocol notfications.

Person
[redacted]
[redacted]

8. Emergency Contacts

* Add at least one person authorized to act in an animal emergency if the Principal Investigator is not available. This person must understand the research and be able to answer questions in the PI's absence.

Person
[redacted]

Funding

Identify all funding sources that support your protocol. If you have questions about grant-protocol congruence, email or submit the **Congruence Review Request Form** to **congruence@rarc.wisc.edu**.

1. Research and Sponsored Program (RSP) Managed Funding

Add any grants or contracts that are funding this project (federal or non-federal).

PI Name	Award Number (MSN #)	Project Title	Sponsor Reference Number	Project ID	Sponsor (Source)	Congruence Determination	Reporting Required
[redacted]	MSN231246	Determining DISSUB survival rates of 90 kg sheep rescued using SRDRS standard operating procedures	N0002420C4303	AAH6262	DOD, NAVY		

2. Other Funding

Add any other funding that is not listed above.

Project Title	PI Name	Award Number (MSN #) / Project ID (PRJXXX)	Start Date	End Date	Grant Status	Sponsor (Source)	Congruence Determination	Reporting Required
There are no items to display								

3. Public Health Service (PHS), NSF, NASA, DOD Funding

See https://en.wikipedia.org/wiki/United States Public Health Service for a list of PHS agencies.

* Are any of the funding sources above (RSP Managed or Other Funding) directly from or subawards from NIH (or other PHS agencies), NSF, NASA, or DOD Funding?

○ Yes ◉ **No**

Protocol Type

Biomedical Research, Basic Biology, Teaching and/or Colony Management

For protocols that involve any of the following:

- Basic biological processes, human clinical medicine, or medical trials intended as models of human (not animal) diseases
- Instruction related to topics listed above
- Breeding and colony management practices for animals used in basic biology and biomedical research and teaching
- Wildlife species brought to campus for more than temporary procedures
- The use of horses to teach students veterinary medicine (the prevention, diagnosis and treatment of disease, disorder and injury in non-human animals)

Agricultural Research, Teaching, and/or Herd Management

For protocols that involve any of the following:

- Improving animals' use in production agriculture
- Trials intended to improve animal welfare
- Breeding and herd management practices for animals used in agricultural

research and teaching
- The use of horses to study or teach equine science (the study of the reproduction, physiology, behavior and nutrition of horses)

Wildlife Study with No Housing OR Educational Display Only

Wildlife Study
For protocols that involve:

- Only wildlife
- No Housing

And may also involve:

- Observation or field instruction*
- Modification of animals' environment
- Capture
- Handling
- Use of anesthesia
- Procedures in the field
- Procedures at a campus location for a period lasting NO MORE than 24 hours

Educational Display
For protocols that involve:

- Housing or no housing
- No experimental procedures
- Wildlife and/or domestic/lab animals

*If the study involves no animal handling and no modification of the animals' environment, a protocol requirement may be waived. Contact an **IACUC administrator** for more information.

Other You must consult with an **IACUC administrator** before selecting.

1. Infectious Disease

* Does this protocol include work with infectious disease?

○ Yes ◉ **No**

2. Protocol Type

For help, email arrow help@rarc.wisc.edu.

* What type of protocol are you submitting? Biomedical Research and Basic Biology and/or Teaching and/or Colony Management

VA ACORP

VA researchers must complete the entire UW protocol application to provide answers about procedures and/or housing at UW facilities.

1. VA Status

Indicate if any of the following apply to this study or project. Select all that apply.

There are no items to display

2. Veterans Administration ACORP

* Is your work also described in an approved Veterans Administration Animal Component of Research Protocol (ACORP)?

○ Yes ◉ **No**

Significance and Justification

1. Significance of Research

* Using language that a high school student would understand (avoid technical grant application language), briefly describe the goals of your study including an explanation of how your work will advance knowledge, improve human or animal health, or benefit society. At the end of your response, briefly and in nonscientific language describe how you plan to interpret the collected data to meet the goals of the study.

When a submarine is disabled, (DISSUB) it can take up to seven days to rescue the stranded submariners. The US Navy has several deep-submergence rescue vehicles (DSRV). This vehicle is located on a ship and must be sailed to the site of the rescue. Therefore, depending on where the submarine is disabled this could take up to a week. During this period, the submariners will be subjected to high pressure and potentially oxygen toxicity. The actual time that people can survive in those conditions is unknown. One recent study found that swine subjected to 5 ATM (atmosphere) pressure died within 55 hours, raising doubts about the ability to survive to be rescued. However, in that study carbon dioxide levels were also elevated. The Navy is funding this research in order to conduct a similar study in sheep, which may be a better model of pulmonary toxicity in humans, and to include both low and high carbon dioxide levels. The ultimate goal is to define the different parameters that stranded submariners may find themselves experiencing and determine survivability. This data will then be used to determine when a rescue mission will be search and rescue versus search and recovery.

The goals of our current grant are to address the following milestone issues:

1. 1. Determine the survivability of hyperbaric air exposure at 5 ATA in sheep for the time required to execute a disabled submarine rescue mission (172 hours)
2.
3. 2. Determine the impact of high inspired CO_2 concentrations on the development of pulmonary oxygen toxicity and survivability
4.
5. 3. If hyperbaric air exposure at 5 ATA (Absolute Atmospheric) for 172 hours is survivable, determine incidence and severity of DCS (Decompression Sickness) following oxygen accelerated decompression using the Submarine Rescue Decompression Plan (SRSDP) tables
6.
7. 4. Provide data that may be used to revise decompression tables in the current SRSDP.
8. Studies to address goal 1 and 2 are included in this protocol. The primary hypothesis to be tested is that high inspired CO_2 concentrations during prolonged air exposure at 5 ATA contributes to the development of pulmonary oxygen toxicity, and decreased survivability.

   Goals 3 and 4 will be proposed in a future protocol depending on the results of this study.

   The results of this research should allow the US Navy to develop predictive decision-making algorithms to improve triage of DISSUB rescues, optimize DISSUB rescue, quantify the impact of delays on the likelihood of a successful rescue, and ultimately improve the chances of successfully rescuing submariners from a DISSUB.

2. Justify Use of Animals

* Explain why you must use live vertebrate animals instead of nonanimal alternatives such as computer simulation or in vitro systems.

> The US Submarine Rescue System Decompression Plan (SRSDP) outlines the mobilization of assets and execution of DISSUB rescue. However, many of these theoretically feasible operational scenarios have not been tested in humans or large animal surrogates. The theorized maximum survivable internal pressure for DISSUB rescue is 132 fsw (feet sea water); however, the effects of pulmonary oxygen toxicity at this depth for the duration of rescue are unknown. Hall and other demonstrated that swine experience significant pulmonary edema after 96 hours at 100% surface oxygen, which is equivalent in partial pressure to breathing air at 132 fsw. The effects of pulmonary edema on nitrogen off-gassing and decompression sickness (DCS) are unknown. In the proposed study we will test the aforementioned knowledge gaps, focusing on the survivability of breathing air at 132 fsw, and determine the impact of high inspired CO2 concentrations on the development of pulmonary oxygen toxicity.
>
> There are no in vitro systems or computer simulations that can accurately model complex physiologic changes that take place in the environment created in this study.

Experimental Narrative

1. Experimental Narrative Summary

If you are unsure if your study-specific husbandry practices are different from the standards provided by the vivarium staff, consult with a RARC research animal veterinarian, WNPRC veterinarian, or the supervisor of the animal facility.

* In language that scientific colleagues outside your discipline would understand, provide a global, chronological summary of your experiments that focuses on the experience of the animals from initial assignment to final disposition. Briefly outline all proposed surgeries, non-surgical procedures, and other manipulations. Do Not Include: breeding schemes, blood draws, housing arrangements, complete surgical descriptions, euthanasia methods, drug doses, drug routes, or other standard practices.

Animals: Prior to assignment to the study, sheep will be given a chest radiograph and physical examination. Animals will be excluded from the study if there is evidence of preexisting pulmonary disease.

We propose to use 12 female sheep weighing approximately 90 kg. Animals will be divided into 2 groups of 6, based on inspired CO_2 concentrations, otherwise the animals will be treated identically.

Group 1 (n 6) will undergo the stimulated DISSUB rescue with 1.5% inspired CO_2.

Group 2 (n 6) will undergo the stimulated DISSUB rescue with 0% inspired CO_2.

Animals will be studies in pairs with the CO_2 concentrations randomly assigned.

Monitoring: All animals will be monitored continuously during the stimulated DISSUB rescue. (See attached Monitoring Form which outlines what is recorded and the increased observation times when the animals starts showing any signs of respiratory dysfunction). Physiologic measures will include respiratory rate, electrocardiography (ECG), and heart rate (AD Instruments Dual Bio Eight Channel 16 bit, 200 kHz data acquisition system and LabScribe 8 Software (iWorx, Dover, NH)). The sheep will also be under continuous closed-circuit video monitoring. The operational air filtration system, with Lab View (National Instruments, Austin, TX) continuously monitors chamber pressure, O_2, CO_2 and N_2 concentrations, humidity, and temperature. All data will be compiled into Excel spreadsheets then imported into Graphpad Prism 5 for statistical analysis. After euthanasia the animal will be prepared for necropsy and histopathological lungs sections. The primary outcome will be clinical diagnosis of pulmonary oxygen toxicity. Carbon dioxide levels will be limited to 1.5% or 0% surface equivalent value by controlled gas flow into the chamber.

Dive Profile: Due to the temperament of the sheep, we will use a pair of animal per experiment. Before experiment, we will insert an IV catheter, apply three ECG pads, and place a respiratory belt on the sheep. They will be placed in the chamber and tied with a halter and lead rope in order to prevent the ECG lines from becoming entangled. They will have free access to food (soy bean pellets) and water, and can lie down within that space. Sheep will be acclimated to halter and lead rope restraint prior to the experiment.

The operational air filtration system, with Lab View (National Instruments, Austin, TX) continuously monitors pressure, O_2, CO_2 and N_2 concentrations, Humidity, and Temperature which is recorded on the diving computer.

The feces/waste will removed pneumatically with water from the bottom of the chamber using a garden hose connected to sewer outlet. The grated floor has holes, so that feces and urine will go through it to the bottom ensuring that the sheep will not be laying in feces.

Once the animals are situated and all monitoring equipment tested and operational the animals will descend at 30 fsw/min to a depth of 132 fsw (5 ATA). They will remain at depth up to 172 hours or until one sheep meets the criteria for euthanasia based on alternative endpoints (see complication section and monitoring form). At that point, the remote euthanasia injection device will be activated and both sheep will be euthanized. The ECG monitor will confirm cardiac arrest. The chamber will be brought up to surface pressure (which will take approximately 4 minutes). Death will be confirmed by lack of heart rate and corneal reflex and animals will be submitted for pathology examination.

Necropsy and Histological Analysis: A necropsy will be performed by a pathologist as soon as possible following euthanasia or death. Body fat will be indexed by the thickness of subcutaneous fat between the 12th and 13th ribs, and by rump fat pad thickness also measured bilaterally and recorded. In gross pathology of lethal outcomes, major organs will be examined, and lung specimens will be harvested to evaluate potential lung hemorrhage, and perivascular and interlobular lung edema. Tissue samples will be harvested and fixed in 10% neutral buffered formalin for histopathologic analysis (i.e., H&E, Immunohistochemistry). Brains and spinal cords will be examined for hemorrhage,

especially in cases of clinical CNS dysfunction. Spinal cords will be fixed and stored for further histologic and MRI examination.

2. Research Cores

* Do you plan to transfer animals for services under a research core protocol?

○ Yes ◉ **No**

3. Supporting Publications or Manuscripts

Do not list standard husbandry references.

List the title/name of manuscripts, abstracts, or other references supporting your research that the IACUC may find helpful in evaluating this protocol.

Lanphier EH, Lehner CE. "Animal models in decompression". In : Man in the Sea, edited by Y. C. Lin and K. K. Shida. San Pedro, CA: Best, Vol. I, 273-295, 1990

Lehner CE, Ball R, Gummin DD, Lanphier EH, Nordheim EV, Crump PM. "Large animal model of human decompression sickness: Sheep database and preliminary analysis. Bethesda, MD: Naval Medical Research Institute, 1997

Ball R, Lehner CE, Parker EC. "Predicting risk of decompression sickness in humans from outcomes in sheep". J. Appl. Physiol. 86(6): 1920-1929, 1999

Sobakin AS, Lehner CE, Wilson MA, Gendron-Fitzpatrick AP, Sauder AM, Eldridge MW. "Dysbaric osteonecrosis in UW sheep DISSUB trials after a 3-hours oxygen pre-breathe before dropout decompression". The Journal of Undersea and Hyperbaric Medicine. Vol. 35, No. 4, 251-252, 2008.

Hutchinson EB, Sobakin AS, Meyerand ME, Eldridge MW, Ferrazzano P. "Diffusion tensor MRI of spinal decompression sickness. The Journal of Undersea and Hyperbaric Medicine. Vol. 40, No. 1, 23 31, 2013

4. Summary Files

Attach file(s) with timelines, illustrations, figures, or other supplemental information that provides an overview of the protocol. Do not attach copies of grant applications.

Proposed remote injection system

Duplication

Animal welfare regulations do not allow unnecessary duplication of previous experiments.

1. Experiment Duplication

* Do the proposed activities duplicate previous work?

- ○ Yes
- ◉ **No**
- ○ Not Applicable - This is a teaching activity involving different student groups

Selected Species

Questions regarding each species can be found in the Species Details section of the protocol.

Click on the Species Details button next to the species you would like to work on. When you are finished answering questions for all species, click Continue or save and exit. You can exit before answering all questions and return later to finish.

1. Species Details

To add additional species not shown below, check the box:

> No

Species Details	Species	Max. Number	Surgery?	MSS?	Breeding?	GM?	USDA Code	Print	Complete?
	Domestic sheep	12	no		no	no	E		

Species Details

Select Study Team

1. Study Team

For help, email arrow help@rarc.wisc.edu.

* Add all research personnel, including the PI, who will work with a species under this protocol. Do NOT include animal facility supervisors, professional animal care staff, rotating students, or research animal veterinary staff. DO add protocol writers and email contacts if they will work with a species.

	Name	Office phone	Lab phone	Cell phone	Email
View	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

2. Study Team Groups

List team groupings that will work on this protocol (e.g., 4th year veterinary students, BRMS Breeding Core, SPI). Do not name individuals or include any assignments.

> *No Answer Provided*

3. PI Oversight

If the PI (him or herself) will not be handling or working with a live species, explain how the PI will provide the oversight necessary for compliance with animal program regulations and requirements.

> *No Answer Provided*

4. Supervisor/Trainer for Staff

* Please state who will train and supervise study team members.

> [redacted]

5. Confirm Training

For assistance, contact [redacted]

* Confirm that all study team members have completed the Animal Contact Risk Questionnaire and are medically cleared to handle animals.

> Confirm

Assignments and Qualifications

1. Study Team Member Assignments

For help email, arrow help@rarc.wisc.edu

Click 'Add' below to associate each team member with a species and/or a procedure. Each member must be associated with at least one species and each procedure must be associated with at least one member.

Name	[redacted]
Species	Domestic Sheep
Surgeries	*No value entered*
RARC	Animal User Orientation - 2019-10-21

View

Classes	Sheep Training - 2017-12-19 Rat Training - 2017-02-21 Animal User Orientation - 2014-10-27
EHS/UHS Training	Animal Contact Risk Questionnaire - 1/22/2023 Risk Communication in Animal Facilities - 3/11/2023 Safety for Personnel with Animal Contact - 3/11/2025
Education	MS 2018 University of Wisconsin - Madison BS 1998 Upper Iowa University AS 1983 Herzing University CHT 2005 (Certified Hyperbaric Technician), International Board of Undersea Medicine (IBUM), Hyperbarics International, Key Largo FL
Experience	No experience with Rats. I will be instructed by [redacted] in animal handling.
Painful nonsurgical procedures	Hyperbaric exposure (the dive)
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	Jugular catheter placement
Transport Method Assignment	*No value entered*

Name	[redacted]
Species	Domestic Sheep
Surgeries	*No value entered*
RARC Classes	Animal User Orientation - 2018-11-28 Sheep Training - 2017-12-19 Rat Training - 2015-06-17 Animal User Orientation - 2013-11-27 Animal User Orientation - 2008-12-02
EHS/UHS Training	Animal Contact Risk Questionnaire - Expired Risk Communication in Animal Facilities - Expired Safety for Personnel with Animal Contact - 3/11/2025

View

Education	Education University of Wisconsin, B.S. (1976) Certification Basic Hyperbaric chamber operation, 2014, ATMO, San Antonio, TX Safety Course for hyperbaric chambers, 2014, ATMO San Antonio,TX
Experience	Sheep. UW course on handling. Experience with previous experiments on sheep. Rat. UW course on handling. Limited experience with handling rats.
Painful nonsurgical procedures	Hyperbaric exposure (the dive)
Physical euthanasia methods	*No value entered*
Anesthesia Analgesia Sedation Assignment	Jugular catheter placement
Transport Method Assignment	*No value entered*

Name	[redacted]
Species	Domestic Sheep
Surgeries	*No value entered*
RARC Classes	Animal User Orientation - 2018-06-19 Animal User Orientation - 2013-06-25 Animal User Orientation - 2008-07-23 Animal User Orientation - 2005-08-18 Surgery Fundamentals - 2005-02-01
EHS/UHS Training	Animal Contact Risk Questionnaire - 5/5/2023 Risk Communication in Animal Facilities - 2/24/2023

		Safety for Personnel with Animal Contact - Expired
View	Education	DEGREES School of Veterinary Medicine, Saratov State University, USSR, DVM, 1990. Institute of Veterinary Virology and Microbiology, Moscow, Russia, PhD, 1994. PROFESSIONAL MEMBERSHIP Undersea and Hyperbaric Medical Society
	Experience	Dairy cattle. Worked on dairy farm as dairy managment specialist from 1999 to 2005. Procedures include blood drawing, IV, IM, AI, milk fever treatment, calfing, vacctination, ect. Sheep. Experience since 2005. Familiar with physical examination, blood draws, IV, IM injections, intubation, anasthesia, bone scan, necropsy, sampling lungs, spinal cord and bran tissue.
	Painful nonsurgical procedures	*No value entered*
	Physical euthanasia methods	*No value entered*
	Anesthesia Analgesia Sedation Assignment	Jugular catheter placement, xylazine for deep sedation prior to euthanasia
	Transport Method Assignment	*No value entered*

2. Other Relevant Experience or Training

Include any protocol-specific experience and/or relevant training for a given study team member that is not found above.

> RARC trainers or veterinarians will provide additional training to all personal that will be euthanizing the sheep. This will include how to inject substances into a catheter and how to inject solutions into the jugular vein if the catheter is not patent.

Occupational Health

Use of hazardous materials requires separate review and approval by EH&S. The Principal Investigator is responsible for obtaining all relevant approval(s) prior to initiating work with hazardous materials.

1. Occupational Hazards

If you have any questions regarding this section, contact biosafety@fpm.wisc.edu.

* Are any of the following used in the research involving live animals under this application? Check all that apply:

- ☐ Biological hazards (zoonotic agents, human or animal pathogens, human cells, prions, etc.)
- ☐ Chemical hazards (carcinogens, flammables, highly reactive, corrosives, etc.)
- ☐ Physical hazards (UV light, magnetic fields, noise, electric shock, temperature, etc.)
- ☐ Radiation and/or radioactive materials (administration of radionuclides, etc.)
- ☑ **Other hazards (zoonotic agents, BSL1 agents that do not require a biosafety protocol, farm work safety precautions, other.)**
- ☐ NONE. None of the hazards listed above apply to research performed on living animals under this application.

Other Hazards

Describe all other hazards that the animal work in this protocol may pose to study team members or animal care staff and the precautions to be taken to protect personnel.

1. Zoonotic Agents

If a biological hazard was not identified in the Substance Administration section because it is not administered to the animal but it is a known or potential biohazard by the animal (such as Monkey B virus), list those agents below.

There are no items to display

2. Biosafety Level 1 Agents

Details on biosafety level 1 agents should be listed in the table below.

There are no items to display

3. Farm Safety Precautions

Select all farm safety precautions that apply to this protocol.

There are no items to display

4. Other Hazards

Please list any other hazards not described previously.

> Zoonotic Safety.
> Study animals are selected from [redacted], which is monitored for exposure to Coxiella burnetti, the bacteria that causes Q fever. However, to further minimize risk, investigators will always wear a lab coat, gloves and shoe covers when working with sheep to avoid exposure to potential zoonosis such as Q fever. Additionally, because we will not use pregnant sheep as test subjects, the risk of exposure to Q fever is considered low.

Species: Domestic sheep

Justify Species Choice

1. Species or Group Choice Justification

* Explain why you chose this species or target group.

> The US Navy's Submarine Rescue System (SRS) is designed for Disable Submarine (DISSUB) rescues up to an internal pressure of 5 ATA or 132 fsw. The US Submarine Rescue System Decompression Plan (SRSDP) outlines the mobilization of assets and execution of DISSUB rescue, however, the survivability of a prolonged 5 ATA air exposure is unknown, and the anatomy of sheep make this species a preferred model for studying how this exposure can affect humans. Limited data suggest that prolonged 5 ATA air exposure induces pulmonary oxygen toxicity, with distal airway injury and pulmonary edema resulting in pulmonary gas exchange dysfunction. Recently, Hall and colleagues (Hall, Navy Completion Report, 2019, see Appendix/Other for complete report), at the Naval Medical Research Center (NMRC) conducted a DISSUB simulation study, using a 70 kg swine model (n 6), designed to determine whether DISSUB casualties exposed to 5 ATA air could survive long enough to be rescued using current rescue procedures and times (Hall, Navy Completion Report, 2019). All swine exposed to 5 ATA died prior to the projected time to first rescue (72 hrs). The mean latency of death was 52.15 ±1.69 hrs. This very small variance in the latency suggests an exposure to hyperbaric air at 5 ATA in the presence of elevated CO2 is incompatible with life even prior to the theoretical first rescue time (Hall, Navy Completion Report, 2019). These findings are deeply concerning and more experiments on different animal model are needed in order to improve DISSUB rescue planning and execution and to determine the inherent risks associated with prolonged time at pressure that would be experienced under a DISSUB scenario lasting 172 hours.
>
> We would like to use the University of Wisconsin (UW) sheep model (90 kg, n 12) to evaluate survivability, risk and preventive measures during a simulated DISSUB rescue scenario at 5 ATA lasting up to 172 hours. The primary objective is to determine the survivability rate and pulmonary oxygen toxicity of sheep exposed to hyperbaric air at 5 ATA for up to 172 hours in the presence of controlled CO2 concentrations of 1.5 % and 0%. The investigator is familiar with sheep behavior, and experienced at observing subtle clinical signs of pulmonary oxygen toxicity and cardiopulmonary dysfunction.

Number of Animals

1. Maximum 3-year Total

* What is the maximum number of this species that you will use during your protocol's three-year period?
Include control and replacement, breeding colony, preweaned, and euthanized animals.

> 12

2. Animal Number Justification

* Provide a justification for the maximum number of animals requested.
For renewals, provide an updated justification for the animals you require for the next three years.

Statistical considerations require that an adequate number of animals be used in the experimental design of each study to provide:
• Sufficient power to adequately test hypothesized differences,
• An efficient experimental design to avoid the excessive, profligate use of animals.

[redacted] a statistician in the Department of Statistics, was consulted to determine the appropriate sample size and complete the power analysis calculations. Based on group differences from extensive previous work, [redacted] determined that when using a significance level of 0.05, a sample size of n 6 in each group will give ~80% power to detect a difference of about 1 full grade for each of the three clinical outcomes. The minimum number of animals needed to achieve statistically significant data is requested.

Group 1 (n 6) will undergo the stimulated DISSUB rescue with 1.5% inspired CO_2.

Group 2 (n 6) will undergo the stimulated DISSUB rescue with 0% inspired CO_2.

3. Justifications and/or Experience

See policy UW-4131, Justification of Numbers, for guidance and examples of acceptable justifications.

Provide a statistical justification or cite your past experience.

> *No Answer Provided*

4. Upload Number Documentation

Attach file(s) that support your determination of animal numbers. If

possible, use tables to organize your information.

There are no items to display

Bio Species Source

1. Species Source

Animals arriving from outside the main UW-Madison campus will require a time period of acclimation before use. For details, see policy UW-4106 ,Acclimation After Transport.

* Check all sources that apply for this species.

☑	**Investigator at UW-Madison / including another protocol held by PI (check for maximum flexibility in animal transfers)**
☑	**Approved vendor** [redacted]
☐	Bred under this protocol
☐	Investigator at non-UW Madison institution [redacted]
☐	Unapproved vendor
☐	Capture or collection from wild (free-living) population
☑	**Herd, flock, etc**
☐	Client/privately owned animals
☐	Other

Prior Use

Animals that have undergone a major surgical procedure, permanent physiologic alteration, or substantial impairment on a previous protocol are not eligible for major surgical procedures on subsequent protocols.

1. Prior Use of Animals

* Were any of these animals used in another protocol?

◉ ○

Yes No

1.1. Prior Use Description

* Describe previous nutritional manipulations, blood draws, administered drugs or other materials, or any other past manipulations, and explain how you determined that the animals' assignment to past projects will not compromise your research or the animals' health.

> Animals obtained from [redacted] will have been assigned to the herd maintenance protocol A005929-R01. Only routine husbandry manipulations (no experimental work) are performed under this protocol. Only animals in good health as evaluated by a research animal veterinarian with no carry over effects will be used in the present protocol. Following complications with obese sheep and one with a pre-existing lung condition, we will select animals of small to moderate mass (70-90 kg) and evaluate (in consultation with RARC veterinarians as necessary) lung function via radiographs.

Breeding and Genetically Modified Y/N

1. Breeding

* Does your protocol design include breeding of this species?

○ Yes ◉ **No**

2. Genetically Modified

* Will any of this species be genetically modified? Include animals modified through breeding schemes, purchase of genetically modified animals, or modified using CRISPR-cas9.

○ Yes ◉ **No**

Substance Administration Checklist

Include delivery of materials to animals via injection, infusion, inhalation,

implantation, ingestion of food/water, and other means. Include administration of radionuclides. Include nonstandard diets under all other substances.

1. Substance Type Selection

* If you will administer substances, check all purposes that apply.

☑	**analgesics/anesthetics/sedatives to relieve pain or distress caused by nonsurgical and/or surgical procedures**
☑	**euthanasia substance(s)**
☐	all other substances
☐	I will not administer any substances.

Anesthesia/Analgesia/Sedation

Used to relieve pain or distress an animal may experience as a result of the procedures and manipulations described in this species/group. For guidance on organizing information, click on the help icon above.

1. Anesthesia/Analgesia/Sedation Details

* Provide details for any anesthesia/analgesia/sedation substance or regimen you will use.

View

Name	Jugular catheter placement
Drugs and Compounds	Lidocaine Hydrochloride Injectable - 2%
Description	1 - 5 ml SQ once
Monitoring Plan	This drug will be given prior to jugular catheter placement to provide local anesthesia at the site of placement.

Name	xylazine for deep sedation prior to euthanasia
Drugs and Compounds	xylazine (20 mg/ml)

View

Description	0.1 to 0.2 mg/kg IM once
Monitoring Plan	This drug will be given only in the event that the remote euthanasia injection device malfunctioned. If the animal was struggling in the chamber when resurfaced this drug could sedate the animal so the technician could safely administer the euthanasia solution via the catheter or the other jugular vein.

View

Name	xylazine for IV catheter placement and for x-rays
Drugs and Compounds	xylazine (20mg/ml)
Description	0.05 - 0.075 mg/kg IM or IV
Monitoring Plan	This dose will be used for calming the sheep during the IV procedure and the x-ray procedure. This dose generally will leave the sheep in a sedated standing position. If the sedation causes recumbency the drug can be reversed with atipamezole.

Euthanasia Substance

If a substance is used to euthanize this species, it should be entered here. Include CO_2.

1. Euthanasia Substance Details

* Provide details on each euthanasia substance you will use.

View

Name	Beuthanasia-D solution or other commercially available euthanasia solution.
Drugs or Compounds	A nonsterile solution containing pentobarbital sodium (390mg/ml) and phenytoin sodium (50mg/ml) as the active ingredients.
Euthanasia Procedure Description	IV, greater than or equal to 1 ml for each 10 pounds of body weight.

Special Substances Checklist

1. Special Substances Selection

* If you are using any special substances, select all that apply.

☐	cells, cell lines, tissues, or tissue products (animal and/or human)
☐	complete Freund's adjuvant (CFA)
☑	**controlled substances (requiring DEA and sometimes SUA registration)**
☐	nonpharmaceutical-grade compounds
☐	paralytic agents
☐	none of the above

Controlled Substances

Controlled substances are drugs regulated by the Drug Enforcement Administration (DEA) and Wisconsin's Controlled Substances Board, which issue Special Use Authorizations (SUAs) for research use of controlled substances by DEA registrants. Get more information on the **RARC Controlled Substances page**.

1. Controlled Substances Selection

* Check all regimens that contain controlled substances.

	Regimen/Substance Name	Drugs or Compounds	Species
☑	Beuthanasia-D solution or other commercially available euthanasia solution.	**A nonsterile solution containing pentobarbital sodium (390mg/ml) and phenytoin sodium (50mg/ml) as the active ingredients.**	**Domestic sheep**
☐	Jugular catheter placement	Lidocaine Hydrochloride Injectable - 2%	Domestic sheep
☐	xylazine for deep sedation prior to euthanasia	xylazine (20 mg/ml)	Domestic sheep
☐	xylazine for IV catheter placement and for x-rays	xylazine (20mg/ml)	Domestic sheep

2. DEA and SUA Registrant

* Name the DEA registrant and, if required, the SUA registrant for the

controlled substances.

PIs are responsib e for ensuring that a contro ed substances are purchased and dispensed under approved WI SUA (required for most PIs) and DEA registrations.

Obtaining or renewing an SUA can take eight to 12 weeks or more. P ease p an according y.

> WI Special Use Authorization (SUA), Number: [redacted]
> Authorized Individuals: [redacted]
>
> DEA Registration Number: [redacted]
> [redacted]

Nonsurgical Procedures Checklist

1. Nonsurgical Procedures Selection

* Check all types of nonsurgical procedures that will be performed.

☑	**Blood collection** Sampling by nonsurgical procedures
☐	**Food and/or fluid regulation** Applies to scheduled or restricted access to food or fluids for experimental purposes. Do NOT check this box for fasting before sedation or use of anesthesia or for standard presurgical fasting or fluid regulation. Presurgical fasting will be described in Surgery Summary.
☐	**Genotyping/identification**
☑	**Imaging** CT scans, MRIs, ultrasound examinations, X-rays, and other imaging procedures, including those that expose the animal to small amounts of radiation for the purpose of producing a visual image of bodies or processes. If a dye is used for imaging, add details about the dye in Substance Administration.
☐	**Irradiation** Exposure to gamma irradiation and other ionizing radiation for the purpose of affecting animal tissue or physiology. Administration of radionuclides via injection or in food should be described in Substance Administration.
☑	**Physical restraint** Applies to the use of manual or mechanical means to limit some or all of an animal's movement. Does NOT apply to brief procedures that are part of normal handling or husbandry. Does NOT apply to normal wildlife-capturing techniques.
	Other nonsurgical procedures

☑ Applies to a wide range of other experimental manipulations of animals such as behavioral assays, gastric lavage, maze trials, oocyte collection, preference tests, and more.

☐ **I will not perform any nonsurgical procedures.**

Blood Collection

For each blood collection regimen, provide details of the procedure.

1. Blood Collection Details

* The table below lists regimens of blood collection that have been added.

View

Name	Complete Blood Count and Serum Chemistries
Collect Site	Jugular venous
Blood Collection Process	IV jugular vein using vacutainer and tube or from jugular catheter after placement.
Blood Collection Monitoring	*No Value Entered*
Max. Single Draw Vol. (ml)	10-15 ml, before entering chamber
Max. Single Draw Vol. (percent)	1%
# Samples	1
Interval	*No Value Entered*
Blood Terminal?	No
Painful/Distressful?	No
Analgesic/Anesthetic Regimen	*No value entered*

2. Blood Collection Exceed Limits

For any survival blood collection regimens that approach or exceed the maximum collection limits as outlined in the RARC guidelines, describe monitoring and supportive care procedures.

> *No Answer Provided*

3. Blood Collection Justification

Provide justification for survival blood collection regimen limits stated in question #2 or justification for multiple collections in a short period of time.

> *No Answer Provided*

Imaging

Imaging includes X-rays, PET scans, CAT scans, MRIs, etc.

1. Imaging Details

* Provide details for each imaging regimen you will use.

View

Name	Lung evaluation
Modality	X-Ray
Contrast	*No Value Entered*
Duration	1 hour or less
Freq./Animal	1 time only
Description/Monitoring	Sheep will stand and be restrained by technician while radiography is obtained. The radiographs will be evaluated by a board certified radiologist to ensure no abnormalities that may affect lung function.
Painful/Distressful?	No
Analgesia/Anesthesia	xylazine for IV catheter placement and for x-rays

Physical Restraint

Do not include brief (< 15 min) physical restraint that is part of normal animal-handling practices or procedures.

Do not include normal wildlife-capturing techniques.

For more information on the definition of physical restraint, selection of restraint type, acclimating animals to restraint devices, and the monitoring of restrained animals, see **policy UW-4087**, Restraint.

1. Restraint Details

* Provide details for each physical-restraint regimen.

View

Name	Lead ropes attached to halters will be used to keep the sheep from entangling their ECG lines. The sheep will be restrainted from turning completely around. They will however be able to lie down and stand up without impediment.
Max. Duration	Up to 172 hour
Acclimatization	7 days acclimation while in [redacted]. We will start with 15 min restraint using the halter and lead rope and slowly increase the time until they are comfortable in the halter and lead rope for 5 hours.
Monitoring	These animals will be on a video monitor to a computer screen while they are in the chamber. A trained technician will continuously viewed the monitor. If the sheep becomes entrapped in the lead rope the chamber will be slowly decompressed and the sheep will be removed from the chamber and euthanized and a necropsy/histology preformed to see any changes may have occurred during their time under high pressure. If the sheep was entrapped and in distress, both sheep would be euthanized using the remote injection device and the chamber quickly decompressed.
Justification	It is critical that the sheep does not twist the ECG lines that are placed on its body and connected to the external monitor, as well as the IV line attached to the remote euthanasia injection device. Also the sheep must not be allowed to entangle their lead lines around the companion sheep.
Painful/Distressful?	No
Analgesic/Anesthetic Regimen	*No value entered*

2. Restraint Files

Attach file(s) with standard operating procedures or other

supplementary information for physical restraint.

There are no items to display

Other Nonsurgical Procedures

1. Other Nonsurgical Procedures Details

* Provide details for other nonsurgical procedures you will use.

View

Name	Hyperbaric exposure (the dive)
Pre and Post Care and/or Treatment	*No Value Entered*
Description	On the day of the study animals will be weighed and transported to [redacted] Electrocardiogram (ECG) pads will be secured to the skin using vet bond surgical glue and secured on each side using waterproof surgical tape, and respiratory belts will be put on around the chest, and baseline vital signs will be collected. The sheep will be placed in the chamber and dive at a maximum rate of 30 fsw/min to 132 fsw for a planned bottom latency of 172 hours with continuous environmental (e.g. carbon dioxide, temperature, and humidity) and physiologic monitoring. The animals will have continuous, free access to water and food. Animal vital signs and ECG will be recorded during the dive as outlined in the Sheep Documentation/Observation Forms. Every 12 hours, a customized flushing system removed the waste beneath the floor. The chamber will be decompressed at a rate of 30 fsw/min. A laboratory member or appropriate designated person will be on-site to monitor the chamber and the animals 24 hours/day for the entirety of the experiment.
Frequency	1
Painful/Distressful?	Yes
Files	
Analgesic/Anesthetic Regimen	*No value entered*

Name	IV jugular cathether
Pre and Post Care and/or Treatment	*No Value Entered*
Description	The jugular vein is first checked for patency and a 4 x 4 area is clipped over the jugular. The area is prepped

View		with scrub and alcohol until the alcohol wipe is clean. The prepared site is then blocked with lidocaine and a final prep is done. Sterile gloves are then worn. The jugular is occluded and the catheter placed at a 45 degree angle and advanced into the vein. Once blood is flowing from the catheter, the stylet is the held in place and the catheter advanced into the vein. An injection cap is then placed on the catheter and the catheter sutured into place. Then the catheter is flushed with heparinized saline.
	Frequency	Once
	Painful/Distressful?	No
	Files	
	Analgesic/Anesthetic Regimen	Jugular catheter placement, xylazine for IV catheter placement and for x-rays

View	Name	Remote Euthanasia Injection System
	Pre and Post Care and/or Treatment	*No Value Entered*
	Description	This device was developed to euthanize the sheep within the chamber prior to resurfacing when they begin to show signs of oxygen toxicity. Since there will be two sheep in the chamber at a time, two devices were necessary. The device is controlled by a switch wired in from the outside of the chamber. This device injects the euthanasia solution into the sheep using the IV extension lines attached to the jugular catheter.
	Frequency	1
	Painful/Distressful?	No
	Files	
	Analgesic/Anesthetic Regimen	*No value entered*

Surgery Y/N

1. Surgery Performed

Surgical procedures that are initiated on a live animal prior to confirmation of death, such as thoracotomy for terminal perfusion, are considered surgeries.

Not surgery: Fine-needle biopsies, intravitreal or subcutaneous injections, simple catheter insertions. These should be described in Other Nonsurgical Procedures.

* Will major, minor, or nonsurvival surgery be performed on any of

this species?

○ Yes ◉ **No**

Alternatives Search

Review the following procedures and genetic modifications (if applicable) you described that cause more than momentary pain or distress. Then answer the questions that follow to explain how you determined that there weren't less painful or distressful alternatives to the procedures.

Nonsurgical Procedures

Procedure Name	Procedure Type	Anesthesia/Analgesia Regimen
Hyperbaric exposure (the dive)	Other Non Surgical Procedures	*No value entered*

Surgical Procedures

Surgery Title	Survival Procedures	Anesthesia/Analgesia Regimen

There are no items to display

1. Alternatives Databases

* List one or two databases you searched (e.g., AltWeb, Biological Abstracts, NORINA, PubMed, etc.) to look for alternatives.

> PubMed

2. Alternatives Years Covered

* What years did your search cover? (yyyy-yyyy)

> 1968 - 2020

3. Alternatives Recent Search

* What was the date of your most recent search?

> 4/15/2021

4. Alternatives Other

What methods did you use beyond database searches to look for alternatives to painful or distressful procedures (e.g. conference attendance, professional expertise, journal articles, training)?

> *No Answer Provided*

5. Alternatives Search Strategy

* Describe your search strategy, including the scientifically relevant keywords you used.

> Disabled submarine decompression, cardiopulmonary decompression sickness, oxygen toxicity, model (all terms sheep, refinement, alternative), CO2 environmental.

6. Alternatives Narrative

* Evaluate the information you've gathered and explain any alternatives or refined methods that cannot be used in this research.

> There is currently no alternative, non-animal model to the UW sheep model. Current computer models are not adequate to meet our experimental goals. We have considered options to deliver medications and/ or euthanasia materials inside the

> chamber. We have tried to run IV lines from the outside of the chamber to a jugular catheter for administration of euthanasia solution. However due to the pressure gradient it was found not to be feasible. We have developed a remote euthanasia injection device.

Complications

In previous sections, you identified the pain and discomfort animals might experience from each procedure. Now consider your procedures from a broader perspective.

1. Potential Complications

* What are the potential complications animals may experience from any of your procedures (e.g., internal bleeding after liver biopsy, Graft Versus Host Disease (GVHD) with transplant) or from any chronic condition resulting from the procedures (e.g., lameness, disease) and how will the complications be managed?

> The potential complications sheep may experience from hyperbaric exposure is pulmonary oxygen toxicity, with clinical signs of cardiopulmonary dysfunction or the chokes.
>
> These sheep will be monitored continuously while in the chamber using video monitoring. The Sheep Documentation/Observation Form (attached to this protocol in another section) outlines the documentation of this monitoring and also outlines when increased documentation and euthanasia will occur.
>
> The mental status, eating/drinking, heart rate, respiratory rate, ability to stand or lie down, and ECG will be documented every hour until the heart rate or respiratory rate increase at which time monitoring will go to every 15 minutes until the sheep begins to pant or cough.
>
> We anticipate the sheep to develop clinical signs of oxygen toxicity which include: increased respiratory rate, coughing, potential seizures and death. Our goal is to set the following parameters that if the sheep is panting with increased abdominal effort for more than 30 minutes, or coughing every 5 minutes, or open mouth breathing, collapse, foaming at mouth or seizures that the animals will be immediately euthanized using the remote euthanasia injection device system.
>
> However it is possible that the device malfunctions, or the catheter has become clotted with blood and the device is unable to administer the euthanasia solution. In that case the chamber would be immediately decompressed so the animal could quickly be euthanized by jugular injection via a technician however during that 4 minute decompression time the animal could die.
>
> Also, severe lethargy or inability to rise or ambulate are conditions that would

indicate that an animal would not be able to reach for food/water. Animals will be euthanized within 4 hours of not being able to rise.

Since the sheep will be euthanized immediately with injectable euthanasia solution and submitted for pathological evaluation, we will not apply re-compression treatment procedures.

If sheep becomes tangled in the lead rope, we will decompress slowly, however if sheep show signs of discomfort during the depressurization they will be euthanized. If the sheep have been under pressure for more than 24 hours, then they will be necropsied and have histology done on the tissues to learn if any pathology has occurring during the time they were under pressure.

Water for the sheep will be added automatically. If the system will not be able to delivery water we will stop the experiment. Enough food has been calculated to provide nutrition for 7 days. If they ran out of food, the experiment would be stopped.

The chamber is temperature controlled so that labored breathing would not be due to increases in ambient temperature within the closed chamber.

If equipment malfunctions in a manner that affects the sheep's welfare or the experiment, then the experiment will be stopped and the sheep either euthanized, or resurfaced if they show no signs of discomfort during the depressurization.

2. Unrelieved Pain or Distress

Will treatment for pain or distress be withheld from any animals of this species?

(•) **Yes** ( ) No

2.1. Unrelieved Justification

* Provide scientific justification for why pain or distress will not be relieved.

We need to simulate actual submarine conditions likely faced by submariners in these situations. Euthanasia will occur at the outlined endpoints stated in the monitoring form and in the complication section. We plan to euthanize the animals in the chamber when signs of oxygen toxicity become present. However if the remote euthanasia injection device system malfunctions, or the catheter becomes clotted, we would need to decompress the chamber in order to euthanize the animal. However it will take 4 minutes to decompress the chamber, so the animal may die during that time period. Therefore this protocol is classified as E (treatment for pain and distress will not be given). However our goal is to euthanize the animals in the chamber when signs of oxygen toxicity become evident.

USDA Designation

The United States Department of Agriculture (USDA) established the following B-E categories based on levels of pain, discomfort, and distress associated with procedures.

1. USDA Designation Code

* Choose the highest category of pain/distress that this species will experience as part of this protocol.

○	B	Animals bred or held for use in teaching, testing, experiments, research, or surgery but not used for such purposes
○	C	Teaching, research, experiments or tests conducted that involve no pain or distress that require use of analgesics
○	D	Experiments, teaching, research, surgery or tests conducted that involve accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic or tranquilizing drugs or palliative measures are used (including surgery or procedures under anesthesia that without the anesthesia would be painful)
◉	**E**	**Teaching, experiments, research, surgery or tests conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic or tranquilizing drugs are not used because they would adversely affect the procedures, results or interpretation of the teaching, research, experiments, surgery or tests**
○	Not USDA-covered activity or species	USDA animal welfare regulations do not apply to the use of this species as described in this protocol

Endpoints/Euthanasia Methods

The RARC veterinary staff has recommendations for euthanizing the most commonly used species on campus. Your euthanasia plans must follow these recommendations unless your alternative method is scientifically justified and approved by your IACUC. Click on the blue question mark icon to view these recommendations and the AVMA Guidelines for the Euthanasia of Animals.

1. Criteria for Anticipated Euthanasia

What are your study endpoints?

> Euthanasia of animals, or rapid death before euthanasia can be performed (as detailed on the Complications page), during 48 -172 h of hyperbaric exposure at 5 ATA (132 fsw) of pressure is expected. We will be monitoring for clinical signs discussed previously that indicate the sheep is developing oxygen toxicity. Once we determine that the clinical signs are severe enough that the sheep would just continue to deteriorate we will euthanize the animals within the chambers. However as discussed previously, if the remote device malfunctions or the catheter has clotted, then the chamber would need 4 minutes to depressurize so that staff members could enter to administer euthanasia solution. It could be possible during those 4 minutes of depressurizing that the animal may die.
>
> We anticipate the sheep to develop clinical signs of oxygen toxicity which include: increased respiratory rate, coughing, potential seizures and death. Our goal is to set the following parameters that if the sheep is panting with increased abdominal effort for more than 30 minutes, or coughing every 5 minutes, or open mouth breathing, collapse, foaming at mouth or seizures that the animals will be immediately euthanized using the remote euthanasia injection device system.
>
> These sheep will be monitored continuously while in the chamber using video monitoring. The Sheep Documentation/Observation Form (attached to this protocol in another section) outlines the documentation of this monitoring and also outlines when increased documentation and euthanasia will occur.
>
> Also, severe lethargy or inability to rise or ambulate are conditions that would indicate that an animal would not be able to reach for food/water. Animals will be euthanized within 4 hours of not being able to rise.

2. Criteria for Unanticipated Euthanasia

* For unanticipated events or nonstudy-related health issues, what criteria or clinical signs will you use to determine an unanticipated endpoint for an animal?

> After 48 hours at hyperbaric exposure is a critical time for anticipated euthanasia. We will use such criteria as outlined in experimental narrative and on the Sheep Documentation/Observation Monitoring Form on when to euthanize.
>
> Unanticipated Euthanasia include:
> If sheep becomes tangled in the lead rope, we will decompress slowly and euthanize both sheep. They will be necropsied and have histology done on the tissues to learn if any pathology has occurring during the time they were under pressure. Likewise if there is an equipment malfunction that affects the sheep's welfare or the experiment the experiment will be halted and the sheep resurfaced or euthanized based on time in the chamber and comfort levels during depressurization.
>
> Water for the sheep will be added automatically. If the system will not be able to delivery water we will stop the experiment. Enough food has been calculated to provide nutrition for 7 days. If they ran out of food, the experiment would be

stopped. They will be necropsied and have histology done on the tissues to learn if any pathology has occurring during the time they were under pressure.

During routine husbandry and care, RARC veterinary staff will be notified of any abnormal clinical signs during this period and clinical care will be managed under the supervision of clinical veterinary staff.

3. Plan for Anticipated Euthanasia

Select all applicable euthanasia methods for planned study procedures.

	Regimen/Substance Name	Drugs or Compounds	Species
☑	Beuthanasia-D solution or other commercially available euthanasia solution.	**A nonsterile solution containing pentobarbital sodium (390mg/ml) and phenytoin sodium (50mg/ml) as the active ingredients.**	**Domestic sheep**

4. Plan for Unanticipated Euthanasia

Select all applicable euthanasia methods for unanticipated events or nonstudy-related health issues.

	Regimen/Substance Name	Drugs or Compounds	Species
☑	Beuthanasia-D solution or other commercially available euthanasia solution.	**A nonsterile solution containing pentobarbital sodium (390mg/ml) and phenytoin sodium (50mg/ml) as the active ingredients.**	**Domestic sheep**

5. Plan for Physical Methods of Euthanasia

After discussing with an RARC veterinarian, describe your plan for physical methods of euthanasia.

Name	Description
There are no items to display	

6. Other Euthanasia Methods

Describe other planned and unplanned euthanasia methods not

included above, including euthanasia performed by the RARC veterinary staff.

> *No Answer Provided*

7. Nonstandard Euthanasia Justification

For methods of euthanasia described above that are NOT listed in RARC Veterinary Standards for this species, justify the use of this method.

> *No Answer Provided*

8. Ensure Death

* Describe the methods you'll use to ensure death following euthanasia procedures.

> Death will be confirmed by respiratory and cardiac arrest. If the ECG monitor is on the animal, no electrical activity "flatline" can also be used.

Disposition

Indicate the final arrangements for animals assigned to this protocol.

1. Disposition Plan

* At the end of their assignment in this protocol, animals will be:

- [ ] Made available to other investigators.
- [ ] Returned to a UW colony, herd or flock for other use.
- [ ] Returned to their client-owners.
- [ ] Maintained at a privately owned herd or flock.

- [ ] Made available for adoption. Adoption must be preapproved by a laboratory animal veterinarian.
- [ ] Sold at market.
- [x] **Euthanized.**
- [ ] Other.

2. Consumption

* Is there a possibility that animals or humans will consume your animals or their byproducts at the end of your study?

○ Yes ◉ **No**

Nonstandard Husbandry Checklist

Don't include medically justified, standard pre- or post-anesthetic/surgical exceptions, such as short term withholding of food and water. Describe these in SURGICAL PROCEDURES.

Don't include longer-term food or fluid regulation. Describe these in NONSURGICAL PROCEDURES.

Don't describe the use of wire bottom caging here if non-avian animals will be on wire-bottomed caging for less than 12 hours. That should be included in the EXPERIMENTAL NARRATIVE.

This protocol assumes that social animals (including Nonhuman Primates) may be housed singly for non-experimental reasons (e.g. husbandry management, veterinary clinical management) in accordance with campus policies and SOPs.

Don't check 'Single housing of social species' if the reason for single housing is approved in the **UW-Madison Animal Social Housing and Enrichment Requirements (ASHER)** document. If you are using Nonhuman Primates and are unsure if you should check this box, consult with your research animal veterinarian.

1. Nonstandard Husbandry Selection

* Check ALL non-standard conditions that apply to this species.

- [x] **Housing animals outside dedicated animal facility**
  Animals will be kept for greater than 12 hours for USDA covered animals, or 24 hours for non-USDA covered animals in any location that is not a dedicated animal facility.
- [ ] **Lab staff provide husbandry in facility**
  Laboratory or research staff, rather than professional facility animal-care staff, will provide animal husbandry for a subset of animals housed in facilities.
- [ ] **Single housing of social species**
  Social species are singly housed for periods longer than 12 hours for experimentally-driven reasons. This does not include: clinical reasons, recovery from anesthesia/surgery, social incompatibility, final animal in an experiment, and female rodents near parturition (see ASHER document).
- [ ] **Enrichment withholding**
  Animals are not provided with the minimum required enrichment as outlined in the facility SOP.
- [ ] **Exercise withholding for dogs**
  Dogs are not provided with the minimum exercise as required by the facility SOP.
- [ ] **Ambient Noise**
  Animals will be exposed to white noise that is not part of the standard environmental enrichment for the species.
- [ ] **Nonstandard lighting**
  Animals will be exposed to lighting paradigm of non-standard wavelength, intensity, or altered light/dark.
- [ ] **Vibration**
  Animals will be exposed to vibrations of an amplitude and or frequency known to cause clinical effect.
- [x] **Cleaning/sanitation schedule different than facility standard**
- [ ] **Enclosure smaller or denser than standard for species**
  Animals will be housed in an enclosure that is smaller than the facility standard or at a density higher than the standard for the cage size.
- [ ] **High velocity air**
  Animals will be directly exposed to high velocity air that is not a normal part of their husbandry.
- [ ] **Bare floor (no bedding) with no structure for resting or sleeping**
- [ ] **Wire bottom cage for more than 12 hours (NOT AVIAN)**
- [ ] **Temperature outside recommended range**
  Animals will be exposed to temperatures outside of the normal reference ranges for the species.
- [x] **Other nonstandard housing or husbandry**
  Animals are subject to other non-standard housing or husbandry conditions.
- [ ] **Not applicable**
  There will be no non-standard husbandry for this study.

Housing Outside Facility

Laboratory or research staff, rather than professional facility animal-care staff, will provide animal husbandry in lab housing areas.

1. Lab Housing Justification

* Justify why you will house animals in a laboratory rather than in a facility.

> Animals will be housed in chamber during experiment.

2. Lab Husbandry

* Briefly outline the husbandry lab staff will provide. Describe any departures from the relevant facility SOP.

> Lab staff will provide water and food before experiment. Water can be added during the experiment to ensure it is clean and fresh . Enough food will be added to cover 7 days in the chamber for 2 sheep.

3. Lab Husbandry Time

* Outline the duration of housing and provide the schedule of husbandry that lab staff will provide.

> Up to 172 hours in chamber.

4. Lab Husbandry Files

Attach file(s) with standard operating procedures or other supplementary information for lab husbandry in lab housing.

There are no items to display

Different Cleaning/Sanitation Schedule

1. Different Cleaning/Sanitation Schedule Description

* Describe how your cleaning/sanitation schedule will be different than the facility standard, including the approximate duration of the different standard, and the number of animals you anticipate using.

> The animals will not have any cleaning or sanitation while in chambers. The floor is slatted to remove urine and fecal material so that the sheep will not become soiled. The chamber floor will be flushed every 12 hours with water.

2. Different Cleaning/Sanitation Schedule Additional Monitoring

* Describe the additional monitoring you will provide for animals exposed to different cleaning/sanitation schedule.

> The animals will be continuously monitored while in the chamber.

3. Different Cleaning/Sanitation Schedule Justification

* What is your justification for utilizing a different cleaning/sanitation schedule than the facility standard?

> We will not be able to access the sheep in the chamber while they are under high pressure.

Other Non Standard Husbandry

1. ONSH Description

* Describe any other non-standard husbandry.

> Food is placed in the chamber prior to beginning the experiment. The sheep will have access to the food during the experiment.

2. ONSH Duration

* Outline the duration and schedule of the non-standard husbandry condition.

> Enough food is provided for 7 days per sheep.

3. ONSH Monitoring

* How will you monitor animals under non-standard husbandry conditions?

> video

4. ONSH Justification

* What is your justification for non-standard husbandry conditions?

> Chamber is under high pressure and unable to access animals.

Select Locations

Add all housing and procedure locations for this species. Use only one of the following three questions to add a location.

Add your location in question 1, if it has been approved by the IACUC.
If you will house animals and perform procedures in the same established animal facility:
Type [redacted] in the search box and select from the results. To allow flexibility and avoid possible protocol violations, do not select a specific room.

If you will use space in UW Veterinary Care [redacted]
Type [redacted] in the search box and select from the results. Do

not select a specific room.

If you will use a [redacted] PI laboratory to hold animals and/or perform procedures:
Type the room number in the search box and select from the results.
Include the building module [redacted]Add each room separately; you cannot add room ranges.

Add your location in question 2, if it is a UW-Madison location that you did not find in the search box for question 1.

Add your location in question 3, if it is not controlled by UW-Madison or its affiliates.

1. Current ACUC Approved Locations

Location Common Name	Room Name	Location Type	Committee	Housing Allowed	Procedure Allowed	Surgery Level
[redacted]	[redacted]	lab	SVM	yes	yes	Surgeries Not Allowed
[redacted]	[redacted]	facility	SVM	yes	yes	Most Surgeries Allowed
[redacted]		facility	SVM	yes	yes	Surgeries Not Allowed

2. Locations Not Found under Current ACUC Approved Locations

You must request ACUC approval for these locations.

Building Name	Building Address	Room Name
There are no items to display		

3. Locations Not Controlled by UW-Madison or Its Affiliates

Location	Location Address
There are no items to display	

Select Purpose Of Locations

1. Locations Details

* Click on the name of each selected location. On the pop-up, indicate which of the following procedures and housing will occur at that location. Check all that apply for each location.

Location name	Facility housing	Laboratory housing	Nonsurgical Procedures	Surgical Procedures	Euthanasia
[redacted]	no	yes	Beuthanasia-D solution or other commercially available euthanasia solution., Complete Blood Count and Serum Chemistries, Hyperbaric exposure (the dive), IV jugular cathether, Jugular catheter placement, Lead ropes attached to halters will be used to keep the sheep from entangling their ECG lines. The sheep will be restrainted from turning completely around. They will however be able to lie down and stand up without impediment., Remote Euthanasia Injection	*No value entered*	yes

			System, xylazine for deep sedation prior to euthanasia		
[redacted]	yes	no	*No value entered*	*No value entered*	no
[redacted]	yes	no	Lung evaluation, xylazine for IV catheter placement and for x-rays	*No value entered*	no

Transport

See **policy UW-4099**, Campus Transportation of Laboratory Animals, for guidance on transporting laboratory animals outside the animal facility. A minimum acclimation period is not required for animals intended for use after intra-campus transport or in non-survival procedures; it is however strongly recommended animals receive at least 72 hours post-transport acclimation prior to use in a research protocol. See **policy UW-4106**, Acclimation After Transport.

1. Animal Transport

* Animals will NOT be transported.

○ True ◉ **False**

1.1. Transport Routes

* Check all transport routes you will use.

- ☑ **within, or between adjacent rooms within, a vivarium (animal never leaves the [redacted] - [redacted]**
- ☐ within a building or between connected buildings (animal moves from lab to lab - [redacted]
- ☑ **between buildings [redacted]**
- ☐ to or from field site [redacted]
- ☐

no transport of animals will occur

1.2. Order of Movement

In 2-4 sentences describe animal movement and transport method.

> *No Answer Provided*

1.3. Transport Methods

* How will you transport animals?

☑	**in a dedicated animal transport vehicle or trailer**
☐	hand-carried in a covered cage, in an animal-transport container, or covered on a cart
☐	in a privately owned vehicle, non-dedicated departmental vehicle, or non-dedicated fleet vehicle
☑	**other**

1.3.1. Other Transport Description

* Provide a justification for this transport method as well as the name of department, contact person and/or owner.

> cart

1.4. Transport Files

Upload supplemental information (i.e. SOPs, maps) here.

There are no items to display

End of Species Details

You are done answering questions about this species.

Click on "Species Complete." You will be redirected to the Species start page where you can answer questions about additional species in your protocol or continue to the next section.