INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Animal Use Protocol

Protocol Number: [redacted]
Meeting Date: 7/19/2023

Submitted By: [redacted]
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University Title: [redacted]
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. General Information *Questions 1-5*

1. Type of Protocol

Research

2. Protocol Title

[redacted]

3. Animal Species

Rat - Rattus norvegicus

4. Lay Overview

State the research (or teaching) goals in two or three sentences, using language that can be understood by a lay person. Please avoid technical terms and acronyms.

When diving and breathing gas under water the body takes up additional nitrogen gas. This is due to the higher environmental pressure caused by the water compressing the gas in the lungs. When coming back to the surface (decompression) this gas will be excreted. If the amount of gas is too high there will be problems with decompression sickness (a medical diagnosis) due to creation of gas bubbles in various tissues in the body (similar to shaking a soda bottle that create carbon dioxide bubbles). The medical problems depend on the amount of gas and where the bubbles appear. A bubble in the skin may cause an itch, while a bubble in the brain may cause a stroke or death. This is a problem in recreational, commercial, and military operations. This has been studied for over 100 years and still only empirical methods to dive reasonably safe exist. The exact pathophysiology is not known. Current methods can only detect bubbles in venous blood with ultrasound. No method so far has shown tissue bubbles or gas after compression/decompression. The aim of this project is to develop a new method using radioactive gas to study how the nitrogen gas in air affects the body during compression and decompression. We will administer the gas in a compression chamber that simulates diving (but dry, in a lab). We will study the pathophysiology with compression/decompression with animal experiments and due to the morbidity of such experiments we will use anesthetized animals that will be euthanized after the experiments and before waking up to avoid suffering. The possibility to do this now is due to recent developments in imaging technology that can scan and detect radioactive gases.

Describe how this research (or teaching) will benefit society, advance knowledge, or benefit human or animal health.

Operational procedures are still being developed and decompression sickness is still a major problem and mystery despite 100 years of ongoing research efforts. Our new proposed method aims to develop basic knowledge into the mechanisms and possibly solve this problem so that diving can be done more safely in recreational, commercial and military operations. The extension of this research is to improve human health and safety.

Briefly summarize your research in 2 or 3 sentences. Details will be described below in Question 12.

We will have rats breathing radioactive gas while compressed in a pressure chamber to simulate diving. After that they will be imaged with a PET-camera to measure where in the body the gas was distributed and how it is released after compression/decompression ended. All techniques have been done previously in animals and humans (i.e diving/pressurization, breathing gas, PET-imaging) but our new combination has never been explored. The combination will yield new interesting data.

5. Study Endpoint for Animals

☐ Acute/Terminal (animal never awakens from initial procedure)

☑ *Survival (how long) for*

24 hrs

I. Personnel

Existing Personnel

[redacted]

☐ Principal Investigator ☐ Alternate Contact - Edit Access ☑ No Edit Access

☐ No Animal Contact ☐ Surgery-Survival-Major ☐ Surgery-Survival-Minor ☐ Surgery-Terminal ☐ Injections

☐ Post-op Surgical Care ☐ Surgical Records ☑ Anesthesia ☑ Euthanasia ☐ Breeding Mgmt.

☐ Drug-Diet-Admin ☐ Hazard Administration ☑ Blood/Tissue Collect ☑ Handling/Husbandry ☑ Observation

☑ Noninvasive Testing

Alternate Contact [redacted]

☐ Principal Investigator ☑ Alternate Contact - Edit Access ☐ No Edit Access

☐ No Animal Contact ☐ Surgery-Survival-Major ☐ Surgery-Survival-Minor ☐ Surgery-Terminal ☑ Injections

☐ Post-op Surgical Care ☑ Surgical Records ☑ Anesthesia ☑ Euthanasia ☐ Breeding Mgmt.

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☑ Noninvasive Testing

[redacted]

☐ Principal Investigator ☐ Alternate Contact - Edit Access ☑ No Edit Access

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Principal Investigator [redacted]

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☐ Noninvasive Testing

[redacted]

☐ Principal Investigator ☐ Alternate Contact - Edit Access ☑ No Edit Access

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☐ Noninvasive Testing

[redacted]

☐ Principal Investigator ☐ Alternate Contact - Edit Access ☑ No Edit Access

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☑ Drug-Diet-Admin ☑ Hazard Administration ☑ Blood/Tissue Collect ☑ Handling/Husbandry ☑ Observation

☑ Noninvasive Testing

Alternate Contact [redacted]

☐ Principal Investigator ☑ Alternate Contact - Edit Access ☐ No Edit Access

☑ No Animal Contact ☐ Surgery-Survival-Major ☐ Surgery-Survival-Minor ☐ Surgery-Terminal ☐ Injections

☐ Post-op Surgical Care ☐ Surgical Records ☐ Anesthesia ☐ Euthanasia ☐ Breeding Mgmt.

☐ Drug-Diet-Admin ☐ Hazard Administration ☐ Blood/Tissue Collect ☐ Handling/Husbandry ☐ Observation

☐ Noninvasive Testing

II. Federally Required Information and Assurances *Questions 6-9*

In accordance with Public Law 89-544 (Animal Welfare Act of August 24, 1966), Public Law 91-579 (Animal Welfare Act Amendments of 1970), Public Law 94-279 (Animal Welfare Act Amendments of 1976), Public Law 99-198 (Food Security Act of 1985, Subtitle F - Animal Welfare), Code of Federal Regulations, Title 9, Chapter 1, Subchapter A - Animal Welfare, and the Public Health Service Policy on Humane Care and Use of Animals, the UCSD Institutional Animal Care and Use Committee is required to obtain the following:

6. Why are living animals required for your study?
Why can't you use replacements such as cell culture, computer modeling or other non-animal models? Check all that apply:

- [x] The complexity of the processes being studied cannot be duplicated or modeled in simpler systems
- [x] There is not enough information known about the processes being studied to design nonliving models.
- [ ] Preclinical studies in living animals are required by federal regulations prior to human testing.
- [ ] This is a behavioral, learning or developmental study.

Other

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

- [ ] A large database exists for this species allowing comparisons with previous data.
- [ ] The anatomy, genetics, physiology or behavior of this species is uniquely suited to the study proposed. Describe below.
- [ ] This is the phylogenetically lowest species that provides adequate size, tissue or anatomy for the proposed study.
- [ ] This species provides a particularly good model for duplicating the human situation. Describe below.
- [ ] The results will be directly applicable to the health or care of this species. Describe below.
- [x] Previous studies using this species formed the background of this project.
- [ ] The species has unique features that make it the best choice available for this study. Describe below.

Explanation

Prior animal studies in decompression sickness have primarily used rats and sheep. Using rats for these experiments (instead of alternatives as mice, guinea pigs etc) enables us to copy methods and protocols known to create the desired effect: we need to produce a limited decompression stress due to nitrogen gas uptake and bubble formation. Also, rats have been shown to function well as experimental animals in compression-decompression, i.e they can handle the pressure changes similar to a human subject diving.

8. Duplication of Research

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

9. Consideration of Alternatives to Painful Procedures
Are you:

- [ ] Using a warm-blooded vertebrate species OTHER THAN rats, mice or birds bred for research
- [x] Proposing procedures that fall into the USDA Pain Category D or E (causing more than momentary or slight pain or distress).

V. Animal Requirements for the 3-Year Approval Period *Questions 11-13*

11. Strains/Breeds

Breed (Strain/Genotype)	Genetic Alteration	Phenotype/Health Issues	Age (adult, juv, fetus, etc.)
Sprague Dawley rats	None	N/A	adult

12. Research Plan for the 3-Year Approval Period

The **purpose** of this section is to **provide scientific justification for the requested number of animals**. Reviewers must be able to understand the experimental rationale, research plan, and comprehensive history of the animals. One text box should be used for each study or set of closely related studies. Generally, any animal to be used should be assigned to only one text box. If you will not be breeding animals, click on the REMOVE button for the group "Breeding Colony Maintenance".

The format of your narrative may vary but be sure that it includes the following **required components** to describe each study:

- **Rationale:** What is the purpose of the study? What questions will be addressed? (1-3 sentences).
- **Variables:** What are the dependent and independent variables for the study (e.g. strains of mice, drug administrations, etc)? Lists are OK, even encouraged.
- **Study design and timeline:**
  - Indicate what animals in all groups will experience

- Specify the experimental timeframe and endpoint
- Identify group sizes
  (Do not include procedural details here; procedures should be described in the sections below).

- **Calculation of number of animals and pain/distress categories:** Make number calculations transparent (e.g. number of variables x group sizes) and identify appropriate pain/distress categories (C,D or E). *(Note: Explanation of sample size determination belongs in Question 13)*
  Get detailed help and examples.

Number of Animals in Each Category of Pain/Distress

Study or Experiment Name	C	D	E
1. Exercise and Ultrasound of Venous Gas Embolism Detection	0	33	0

Study Rationale, Variables, Design and Timeline

We will create a situation with differences in amount of blood gas bubbles vs gas in solution in a live animal. This can be performed by recreating a rat experiment published in 2001 where it was shown that that exercise the day before compression/decompression reduce the amount of bubbles to the extent that mortality rates changed significantly. Bubbles were studied by ultrasound then as we will do now as well. The published compression/decompression created vast amounts of bubbles in the control animals after a 45 minute compression. We do need to investigate various amounts of bubbles, methods and equipment to study the effect of various levels of gas in tissues. [redacted].
We will have to test and recreate this including calibration of exercise load, and confirm that this procedure works in the currently available strain of rats etc.
[redacted]

Exercise groups:
The exercise cohort will be placed onto a motorized, rat sized treadmill for [redacted] of exercise [redacted] prior to compression in the chamber. After this exercise they will go back to their regular cage until the next day.

Compression/Decompression, all animals.
Animals will first be placed in the chamber for compression/decompression receiving normal pressurized air. Compression/decompression using the same profile as Wisløff and Brubakk 2001 (again, described in the Other Procedures box) with chamber compression to 700 kPa, for [redacted] or [redacted] minutes. The rats will be awake during this procedure as been done in previous publications.
Following the compression cycle, rats will be taken out of the chamber and be anesthetized (isoflurane will be added to the mixture at 3-5% of the total). They will be shaved and naired over the heart. and loosely restrained on a warmed imaging stage, while still anesthetized. Warmed ultrasound gel will be used to couple the transducer to the animal over the chest, and imaging will occur over the course of 1 to 2 hours. Imaging modes may include normal B-mode, color Doppler mode, or contrast mode. All modes utilize similar energy levels, and all are well below the threshold for mechanical or thermal tissue disruption. Total anesthetization time will be 2-3 hrs. Upon completion of the scan, each rat will be euthanized (while still under anesthesia). Decompression can cause long term damage and pain, thus rats will not be allowed to wake up.

The [redacted] compression/decompression profile has, according to the publication, a 50% rate of mortality (within 1 hour after decompression) in the non-exercised rats and significant differences in bubble load compared to the exercised group. It is believed the bubbles contribute to mortality. We expect this part to verify the old experiments and tune our procedures so we have the right size of rat, the correct exercise intensity, and thus a reproducible method to change the amount of gas bubbles during decompression after compression in a chamber. We hypothesize that the [redacted] Please note that while animals may die from decompression sickness, no animal will ever be conscious after compression has begun, until euthanasia. Any animals that die will be heavily anesthetized, as will all surviving animals until they are euthanized. Since death from decompression sickness can be quick and sudden we are unlikely to be able to consistently intervene and euthanize dying animals before the sickness kills them. Further, the bubbles that lead to death are exactly what we plan to image, so seeing how they develop in dying and surviving animals at very late stages is important.
Due to the individual variation in sensitivity to decompression and bubble formation we project a need for 7 animals in each of the [redacted] with an additional 5 animals (approximately 20% over) if some experiments would have to be repeated or failed due to logistical or technical challenges.

Study or Experiment Name	C	D	E
2. Breathing of radioactive gas in the PET at normal atmospheric pressure.	0	20	0

Study Rationale, Variables, Design and Timeline

We will set up and test the technique of breathing or rebreathing radioactive gas (13N2) on a few rats in normobaric conditions, i.e no compression, no chamber.

Each rat will be anesthetized (with isoflurane) and the gas line will be securely attached to their face via a sealed nosecone or intubated. The rat will then be placed upon the micro-PET (Vista DR from GE Healthcare) gantry bed. The animals will then breathe pure oxygen for 1 hour to off-gas nitrogen (i.e reduce the naturally occurring non-radioactive nitrogen in their body tissues and gas in the lungs. They will be advanced into the imager until their heads rest in the center of the field of view. Rats will be breathing off a rebreather system capable of switching gases for recycling the inert gas while scrubbing carbon dioxide, as well as switching to a separate collection of exhaled gas:

The radioactive 13N2 will be mixed with oxygen to generate "air" with 21% oxygen in a "bag" (lead shielded). From that bag will the rat breathe via a tube that connects to the nose-cone. Isoflurane will be added into this tube. When the rat exhales the gas can go two ways (the experimenter can switch a valve), either will the exhaled gas go to a collection bag were it will be allowed to decay all radioactivity (a few hours) before disposed according to the procedures to handle the isoflurane content. The other option is that the exhaled gas goes back into the rebreatherbag via a scrubber system that absorbs carbon dioxide (standard technology for a respirator system). This version may need a small fan to blow the gas through the scrubber material before going back to the "breathing bag". For extra safety, if the nose-cone would leak some gas, we will have a continuous suction system collecting room air around the rat including any possible leak of 13N2 or isoflurane. All bags and tubes outside the PET-system will be covered /shielded by Lead to contain radiation from the experimenters/researchers. Alternatively, rats will breathe/be ventilated with the gas mix from one bag with the exhaled gas collected in a separate bag (both bags contained within the radioactive shielded cabinet)

The micro-PET scanner will be set to take a set of scans cycling through to image the entire body of the rat, over a course of 20 minutes.

Then ventilation will switch to rebreathing air including 13N2 (and isoflurane) for [redacted] or [redacted] minutes (with oxygen continuously added to keep normoxic gas at 21%O2). Then breathing will switch to inspiring regular air with isoflurane. Expired gas will be collected (containing isoflurane and 13N2). Radioactivity will be monitored on the inspiratory and expiratory side (inhaled and exhaled gas separated) as well as in body in 3 dimensions by the PET. All gas (isoflurane and radioactive nitrogen) will be collected. Upon completion of the scans, each rat will be euthanized, and the carcass kept until all activity has been allowed to decay away.

We expect this part to show that the radioactive gas goes into the rat, and that the signal can be detected appropriately by the PET. We also expect this part to develop calibration procedures, e.g. how many minutes scan time is necessary to yield images over certain body areas.
We will test 3 animals each at [redacted] and [redacted] minutes of breathing radioactive gas for a total of six animals. After deciding what duration gives optimal results, 6 animals will be tested each with air or oxygen prebreathing. Adding 2 more animals for techinchal issues, to reach a total of 20 animals.
After experients have finished the animal will be euthanized while still sedated. The heart will be taken out and then the brain, liver and a piece of muscle tissue. Tissues will be weighted and then measured for radioactivity.

Study or Experiment Name	C	D	E
3. Impact of Exercise on Nitrogen Reservoirs in Pressurized Rats	0	33	0

Study Rationale, Variables, Design and Timeline

Each rat will be anesthetized by isoflurane (or xylazine/ketamine dependent on study 4). Once unconscious, the rat will be placed in the chamber and the anesthesia/radioactive gas line will be securely attached to their face via a sealed nosecone and rebreather device. The chamber will be sealed and pressurized while the animal remains asleep. Breathing of 13N2 will commence at the start of compression and last until decompression is complete. For this experiment we will compress and decompress rats in a specially constructed acrylic chamber inside the PET, breathing 13N2 using a microPET scanner.

[redacted]

Exercise groups:
The exercise cohort will be placed onto a motorized, rat sized treadmill for [redacted] of exercise [redacted] prior to compression in the chamber. After this exercise they will go back to their regular cage until the next day. Thus some rats will exercise, and thus the 24 hrs interval in survival studies. These rats will only exercise and survive, after diving they will be euthanized.

Animals will be placed in the chamber for compression/decompression receiving normal pressurized air. Compression/decompression using the same profile as Wisløff and Brubakk 2001 (again, described in the Other Procedures box) with chamber compression to 700 kPa, for [redacted] or [redacted] minutes. Then they will be anesthetized (isoflurane will be added to the mixture at 3-5% of the total). They will then be shaved and naired over the heart.

After decompression the rats will be imaged with PET and may be imaged with ultrasound and gas distribution measured during 1-2 hours of breathing air (all exhaled gas collected separately). Ultrasound may include normal B-mode, color Doppler mode, or contrast mode. Blood will NOT be sampled. The primary imaging data targets using PET will be the brain and the pelvic area (with hips, large muscles etc.) which are areas that does not move in a sedated animal. PET will yield ventilation studies of the lungs in addition to imaging of stationary tissues. MR imaging will follow the same protocol as the PET imaging, by moving the whole chamber into the MR after radioactivity has decayed beyond what can generate images in the PET. The still anesthetized rats will be scanned to get good images of the relevant body areas, and total scan time will probably be about 20 minutes. We expect this experiment to be the proof of concept of studying gas in decompression, and also, show where the gas is distributed in the body and answer the hypothesis on gas diffusivity and changes in supersaturation tolerance. As in the other experiments' animals will be sacrificed after imaging is concluded.

Due to the individual variation in sensitivity to decompression and bubble formation we project a need for 7 animals in each of the four experimental groups with an additional 5 animals (approximately 20% over) if some experiments would have to be repeated or failed due to logistical or technical challenges.

Experiments/imaging will end while the rats are still sedated. They will be euthanized while sedated.

Study or Experiment Name	C	D	E
4. Assessment of anaesthetic depth in rats under hyperbaric pressure.	0	14	0

Study Rationale, Variables, Design and Timeline

We need to ensure rats remain unconscious at depth to allow other studies (namely 3. Impact of Exercise on Nitrogen Reservoirs in Pressurized Rats) to be done without pain or distress to the rats.

Rats will be sedated using either isoflurane or a ketamine/xylazine cocktail before being placed in a custom-built, rodent-sized hyperbaric chamber. The chamber will then be pressurized to either [redacted] or [redacted] kPa. During isoflurane experiments isoflurane content will be adjusted to maintain the same absolute concentration of isoflurane within the chamber, thereby ensuring anesthesia is maintained. The hyperbaric stimulus will be applied for no longer than 45 min. At this time point the chamber will be flushed with CO_2 to euthanize the rat prior to decompression, thereby preventing any pain that could be caused during decompression. 2 rats will experience each condition (i.e., isoflurane and [redacted] kPa, or ketamine/xylazine and [redacted] kPa). Based on the observed depth of anesthesia 2 more rats will be tested at the combination with the best results to ensure it's effectiveness. This will enable that combination to be used in other studies, thereby minimizing any pain or distress that could occur to the rats.

Throughout the hyperbaric exposure rats can be monitored through the clear acrylic of the hyperbaric chamber, and will be monitored closely for changes in respiration rate, general body response, and eye movement. If there are any signs that anesthetic depth is reducing the chamber will immediately be flushed with CO_2 gas to euthanize the rat before any pain or distress occurs. Following the experimental period the chamber will also be flushed with CO_2 gas to euthanize the rat, thereby preventing any long-term health issues associated with decompression sickness which may occur when decompressing the rat.

Group Pain Category Subtotals and Totals	Subtotal C	Subtotal D	Subtotal E	Total(C+D+E)

0 100 0 100

13. Justification of the number of animals requested. How were the sample size, number of groups, and number of repetitions determined?
Check all that apply.

- [ ] Power analyses indicate that the proposed number of experiments is the lowest required for statistically valid tests of the hypothesis. Describe below.
- [x] The experiments will compare the effects of several independent variables and therefore require many groups or cohorts. Describe below.
- [ ] The outcome measures or phenomena being measured are variable and large sample sizes are necessary for statistically valid sampling. Describe below.
- [ ] Differences from controls are expected to be small, and large sample sizes are necessary to distinguish differences reliably. Describe below.
- [x] The experiments are technically difficult and multiple attempts will be needed to obtain satisfactory data from each experiment. Describe below.
- [ ] These animals will be used to produce antibodies or tissues. Describe the amount of tissue needed and how much is produced from each animal below.
- [x] This is a pilot study to obtain preliminary data to determine if a larger study can be done. Describe below.
- [ ] This is product testing done under FDA guidelines which necessitate using this sample size. Describe below.

Other - Explain in detail below

We have estimated that 5 animals is enough to test conditions without pressure changes and 7 animals when we add the pressure changes. This to account for variation in responses between animals. The study design is to obtain preliminary data to whether gas can be traced in a live animal and whether the variations in amount is detectable with changes due to breathing gas and/or pressure. The additional intervention of exercise 24 hrs prior to diving is also included as a separate variable. The numbers are based on a previous publication (Wisloff and Brubakk) from where we try to recreate the similar diving/exerimental conditions. Due to techincal difficulties with gas delivery we have added a few additional animals (5) to each condition as a reserve.

/. Experimental Details *Questions 14-29*

14. Breeding of Animals

- [x] We will not do Breeding of Animals on this Protocol

15. Behavioral Studies

- [x] We will not do Behavioral Studies on this Protocol

16. In Vivo Blood/In Vivo Fluid/In Vivo Tissue Collection

- [x] We will not do In Vivo Blood/In Vivo Body Fluid/In Vivo Tissue Collection on this Protocol

17. Administration of Anesthetic, Analgesic, Therapeutic and Experimental Compounds

- [ ] We will not administer any compounds to animals on this protocol

Federal regulations require that all compounds administered to animals be pharmaceutical grade if commercially available, including diluents.
Please see:

- Policy on the Use of Non-Pharmaceutical Grade Compounds in Animals
- UCSD Recommended anesthetic and analgesic compounds by species
- Guidelines for the Use of Injectable Drugs in Animals
- Rodent anesthesia equipment

17 A. List all compounds that will be used in/on live animals. Click the Save button to save the current line and add more as necessary.

Experimental Compound

Compound	Dose	Route	Frequency and Duration
13N2	<1% of breathed air	Inhaled	Once, 1-2hours

Possible adverse effects:

None, 13N2 is chemically identical to normal nitrogen in air.

Anesthetic/Euthanasia Compound

Compound	Dose	Route	Frequency and Duration
Carbon Dioxide	95%	inhaled	Once

A) Criteria for assessing depth of anesthesia and, if using inhalant anesthesia, B) description of apparatus for administering Compound (e.g., precision vaporizer):

Carbon dioxide will be used to euthanize rats who show signs of anaesthesia wearing off. The carbon dioxide comes from a pressurized cylinder which enables euthanization without needing to decompress the animal which could lead to pain, especially if done quickly. Carbon dioxide can be readily added, leading to euthanasia within seconds.

Anesthetic/Euthanasia Compound

Compound	Dose	Route	Frequency and Duration
isoflurane	2-5%induction 1-3%maintanence	inhaled	throughout the imaging part of the experiment.

A) Criteria for assessing depth of anesthesia and, if using inhalant anesthesia, B) description of apparatus for administering Compound (e.g., precision vaporizer):

reflexive extremity and eye movement and general body response, heart rate and respiration we use a precision vaporizer

Anesthetic/Euthanasia Compound

Compound	Dose	Route	Frequency and Duration
Ketamine/Xylazine	100 mg/kg and 10 mg/kg	Intraperitoneal Injection	Once for every 30 min assessment

A) Criteria for assessing depth of anesthesia and, if using inhalant anesthesia, B) description of apparatus for administering Compound (e.g., precision vaporizer):

Eye movement, general body response, respiration rate

Anesthetic/Euthanasia Compound

Compound	Dose	Route	Frequency and Duration
Sodium Pentobarbital	150 mg/kg	IP	once

A) Criteria for assessing depth of anesthesia and, if using inhalant anesthesia, B) description of apparatus for administering Compound (e.g., precision vaporizer):

followed by removal of a major organ (or bilateral thoracotomy).

17 B-C. Non-Pharmaceutical Grade Compounds

Federal regulations require that all compounds administered to animals be pharmaceutical grade if commercially available, including diluents.

Please read IACUC Policy 31 on the Use of Non-Pharmaceutical Grade Compounds in Animals before answering the following questions.

17 B. Pharmaceutical grade formulations are widely available for *therapeutic* compounds (anesthetics, analgesics, antibiotics, euthanasia compounds, etc.) that are not used experimentally.

Do you propose to use any *therapeutic* compound on animals that IS NOT pharmaceutical grade?

- ○ Yes
- ◉ No

If YES, name each non-pharmaceutical therapeutic compound from 17 A., provide scientific justification for not using a pharmaceutical grade product and describe how you will assure purity, sterility, and effectiveness of each preparation. If preparation methods vary per compound, please describe the methods to be used for each compound or class of compounds.

17 C. 1. Pharmaceutical grade compounds may or may not be available for *experimental* compounds (test compound, compounds used to induce a model or condition, tracers, etc.).

Do you propose to use any *experimental* compound that IS NOT pharmaceutical grade?

- ○ Yes
- ◉ No

If YES, name each non-pharmaceutical experimental compound from 17 A. and describe how you will assure the purity, sterility, and effectiveness of each of your preparations. If preparation methods vary per compound, please describe the methods to be used for each compound or class of compounds.

17 C. 2. Do any of the *experimental* compounds listed in 17 C. 1. have a pharmaceutical grade preparation that is commercially available?

- ○ Yes
- ◉ No

If YES, name each non-pharmaceutical grade compound from 17 C. 1. and provide scientific justification for not using the pharmaceutical grade preparation(s). If your justification varies per compound, please provide scientific justification for each compound or class of compounds. Example wording for scientific justification can be found in IACUC Policy 31.

18. Paralyzing Compounds

☑ We will not use Paralyzing Compounds on this Protocol

19. Surgery

☑ We will not do Surgery on this Protocol

20. Food or Water Restriction

☑ We will not do Food and Water Restriction on this Protocol

21. Extended Restraint

Do not include brief periods of restriction for blood collection or injection. Please read Policy 27 on Extended Restraint before answering the following questions:

☑ We will not do Extended Restraint on this Protocol

22. Tumors/Neoplasia

☑ We will not do Tumors and Neoplasia on this Protocol

23. Death as an Endpoint

☑ We will not do Death as an Endpoint on this Protocol

24. Arthritis

☑ We will not do Arthritis on this Protocol

25. Adjuvant Use or Polyclonal Antibody Production

☑ We will not do Adjuvant Use or Polyclonal Antibody Production on this Protocol

26. Monoclonal Antibody Production

☑ We will not do Monoclonal Antibody Production on this Protocol

27. Introduction/Injection of biologicals (tissue, cell lines, tumors, stem cells, blood components, body fluids) into rodents

☑ We will not use Biologicals in Rodents

28. Medical Imaging or Irradiation

☐ We will not do Imaging or Irradiation on this Protocol

What is the experimental group (from Question 12) which requires Imaging or Irradiation? Describe the procedure(s), including use of anesthesia or sedation, restraint used, contrast media administered, etc.

For animals that will dive first they will be anesthetized directly after diving before imaging starts.

For animals breathing 13N2: PET imaging will be performed in each rat. Each rat will be anesthetized before put into the compression chamber. Once unconscious, the rat will be placed in the chamber and the anesthesia/radioactive gas line will be securely attached to their face via a sealed nosecone and rebreather device. The chamber will be sealed and pressurized while the animal remains asleep. Following the compression period, the rat will be kept at high pressure for a time and then the pressure will be released. The still unconscious rat will be placed in the microPET scanner (Vista DR, GE Healthcare), and then imaged for 90 minutes. They will will be under general anesthesia (isoflurane) the entire time. Blood will NOT be sampled. The study will require about 2.5 hours of anesthesia time and 90 minutes of ´in-scanner´ time. At the conclusion of scanning animals will be sacrificed.

Animals undergoing ultrasound scans will be anesthetized prior to beginning the scan with isoflurane. They will be shaved and Naired over the heart, then will follow the same procedure of pressurization as the PET scan rats, although they will not receive radioactive nitrogen, instead receiving normal pressurized air. Following the compression cycle, they wil be loosely restrained on a warmed imaging stage, while still anesthetized. Warmed ultrasound gel will be used to couple the transducer to the animal over the chest, and imaging will occur over the course of 1 to 2 hours. Imaging modes may include normal B-mode, color doppler mode, or contrast mode. All modes utilize similar energy levels, and all are well below the threshold for mechanical or thermal tissue disruption. Total anesthetization time will be simialr to that for PET studies. Following these scans, the animals will again be sacrificed.

Animals body temperature is controlled via a KentScientific TempRight monitor that works alongside the RoVent. The animal's core body temperature is maintained using a feedback monitor system to automatically control the far infrared warming pad (Kent Scientific – RightTemp®).

List locations(s) of procedures(s) including building, room number and equipment used.

The PET and Ultrasound studies will be performed in [redacted].

29. Other Procedures

Describe any procedures (such as non-surgical procedures, monitoring, measurements, photography/videography) to be performed on animals that are NOT discussed above.

Treadmill Exercise

Rats will be acclimated/trained to run on a treadmill prior to experiments.
The day of experiment they will be placed on a motorized treadmill for the exercise period. The treadmill will begin with a warm-up period of [redacted], before entering the interval period, when the rats will run a [redacted], followed by [redacted]. These intervals will repeat until the animal has run for a total time of [redacted].

The treadmill will force them to run according to set parameters. The rear of the treadmill to provide shocks if rats do not run willingly. Shocks will be no greater than 2mA. If a rat suffer 3 shocks in one minute it will be considered too tired to run and removed. This exercise protocol follows previously published data, however if we find that the rats we use are unable to reliably run for the established [redacted] period, we will reduce the time, intensity, or both. After this exercise they will go back to their regular cage until the next day.

Pressurization
Anesthetized rats will be put in the chamber at standard pressure (~100kPa). Once sealed inside, the pressure be increased at a rate of 100kPA/min until it reaches [redacted] ([redacted]) and remain at that pressure for either [redacted] or [redacted] minutes. At the conclusion of the diving period, pressure will be released slowly at a rate of 50kPA/min until the chamber returns to 100kPa.

Pressurization in the PET tube (studies 3/4)
Rats will be anesthetized using either isoflurane or xylazine/ketamine and placed inside the PET-compatible hyperbaric chamber. The chamber will then be pressurized to either [redacted] or [redacted] kPa. Rats will remain at pressure for 45 minutes. After this time they will be euthanized using carbon dioxide while still under pressure.e

Intubation will be carried out entirely under isoflurane anesthesia. We will use a Kent Scientific Rat Intubation Platform to perform the procedure. Once unconscious the rat will be moved from the induction box to the platform. There it will be suspended by its upper jaw while a specially formed nose cone is lowered over its snout to continue to

provide and scavenge isoflurane. The platform will be adjusted to properly open the airway and a fiber optic light threaded through a catheter and a safety guide will be advanced down the airway and past the vocal chords to the tracheal bifurcation.

Ventilation: The catheter and light will be removed and an inflatable trachea tube will be advanced in their place. The tube bulb will be inflated and then the tube istself will be connected to the ventilator as described below.

Ventilation will be carried out under deep anesthesia. Once unconscious the rat will receive ocular lubrication and be intubated. They will be connected to a Flexivent Rodent Ventilator by SciReq, which will provide oxygen as well as isoflurane anesthetic. The ventilator will be set to the following parameters:
Tidal volume = 10ml/kg
Respiratory Rate = 90 breaths/min
Positive End Expiratory Pressure (PEEP) = 5 cm H20

Animals will be monitored during ventilation and imaging sessions by watching respiration rate (if off ventilator), toe pinch, and color monitoring. If these prove insufficient to asses animal's health, we will also use a pulse oximeter, and/or a rectal thermometer. A forced warm air system will be used to maintain heat support.
We have visual contact with animals at all times, the chamber has clear acrylic windows.

/I. Potential Animal Pain and Distress/Euthanasia

Questions 30-34

*30. Describe the **potential study-induced problems** the animals might experience (i.e. post-surgical, dehydration, weight loss, pain studies, use of aversive stimuli, extended restraint, retro-orbital bleeding, CFA/IFA injection, arthritis, tumor development, impaired ambulation, other induced disease). Include any health problems due to the phenotype of the animal.*

Imaging itself presents no expected health risks. All these imaging procedures have been done many times before in this imaging facility.

The focus of this study is decompression sickness. Upon release of the increased pressure they are placed under, we expect nitrogen bubbles to form in the joints and blood of these animals. Decompression sickness symptoms include pain, dizziness, disorientation, and in extreme cases, such as those we are studying, potential death. Animals will be anesthetized (with monitoring of anesthesia level) the entire time that decompression sickness is possible, and animals will be euthanized under anesthesia, so they will not experience any of these effects.

Exercised animals may experience some distress from the running period. There will be an electrified grid or small air puffing gun at the rear of the treadmill to encourage the animals to run. Shocked from the grid will be minor and will cause no more discomfort than needed to get the animal running. Discomfort from the physical exertion will likely occur, but we do not plan to run any rat to exhaustion.

Intubation and ventilation could cause distress if the catheter or aerosolizer is extended too far and could injure the lungs. Also, intubation could damage the vocal chords or other parts of the trachea. It could also be extended into the stomach instead of the airway. Damage to the airway or lungs could be painful and cause bleeding. Liquid aerosolized into the lungs could be poorly dispersed, forming droplets that could clog the airways or potentially even drown the animal. The experience of ventilation itself can be unpleasant for the animal if it is improperly anesthetized.

There is a possibility that whilst inside the PET-compatible hyperbaric chamber the anesthesia may wear off. During this time there is no way to increase the anesthetic dose (as it is in a sealed environment), therefore the animal will be closely monitored for signs that anesthetic depth is wearing off, and if so the animal can be euthanized immediately using pressurized carbon dioxide.

*31. For each of the potential problems from Question 30, **describe how any pain and/or distress will be recognized.** Provide the specific clinical signs which will be monitored as well as the frequency of monitoring, including provisions for off hours.*

Any movement, increase in heart rate or jaw tone, pupil reflex, or response to toe pinch will be taken as needed for additional anesthesia. Each animal will be monitored continuously during the whole experiments from start of anesthesia until euthanasia. Animals will be also be monitored for breathing and temperature.

Distress during exercise will be monitored by watching for signs of exhaustion, such as not continuing to run even when 3 shocks are given during one minute as encouragement. A rat that gets 3 shocks will be removed from exercising. We will follow the guidelines for the available threadmill (the ACP has a SOP we can folllow) and adjust speed after our rats performance aiming for [redacted] min of total exercise [redacted].

Some gasping when the catheter is first extended beyond the vocal chords is expected, but excessive gasping, muscle tone or movement will be considered signs of greater distress. Blood on the tip of the catheter when removed will be a clear sign of damage to the airways. Further resistance by the animal during aerosolization will also be watched for. After imaging begins, hot spots of greater signal will indicate poor dispersion of the agent. Similarly, a hot spot outside the lungs, in the gut, will indicate that intubation did not go through the airway, but instead reached the stomach. While the aerosolizing device will occlude breathing while inserted, it should not be inserted for more than a few seconds, so any change in the SPO2 of the animal will likely be a sign that the aerosolized agent is hampering breathing and oxygenation of the animal's blood. Dropping SpO2 after intubation for ventilation will indicate improperly placed breathing tube.

Fighting against the ventilator will be the primary sign of distress. We will observe the animal during its ventilation period for signs of struggle against the machine or other distress. We will also monitor its vitals to ensure it is being properly oxygenated.

In the PET-compatible hyperbaric chamber the rat will have its respiration rate monitored continuously as well as any changes in pupil reflex that may signal that the depth of anesthesia is wearing off.

*32. For each of the potential problems from Question 30, explain **what steps will be taken to alleviate any pain, distress or discomfort** the animals may experience (i.e. call ACP veterinary services, euthanasia, administer analgesia, etc.). If analgesics will not be used, please scientifically justify the reasons.*

Increased anesthesia (isoflurane).

Animals showing signs of distress or exhaustion will have the intensity of their run reduced. If they do not recover with this reduction, exercise will be discontinued.

Struggling against intubation will be met with greater anesthesia. While the experience of being intubated is unpleasant, it should not be enough to cause great distress to the animal. Requiring dramatically greater anesthesia than normal is likely a sign that intubation failed or caused damage to the animal.

Struggling against the ventilator will be met with greater anesthesia. Intubation placement will also be checked if rats begin to struggle.

When the rat is in the PET-compatible hyperbaric chamber, if there is a change in respiration rate of >50% the chamber will be flushed with carbon dioxide to euthanize the animal before awakening, which otherwise may cause pain.

33. Describe in detail criteria for euthanasia:

Any animal exhibiting pain or distress shown by movement that cannot be alleviated by increased anesthetic will be euthanized. All animals will be euthanized at the end of imaging experiments.

Any animal that cannot oxygenate or that shows extreme distress or that shows bleeding from its muzzle that will not stop will be euthanized.

I concur that I will euthanize moribund animals immediately.

34. Method of Euthanasia The Institutional Animal Care and Use Committee policy at Policy 13 Euthanasia requires investigators to follow the AVMA Guidelines for the Euthanasia of Animals: Current Edition unless scientific justification is provided below. Please include ALL methods of euthanasia. Describe the secondary physical method to be used in rodents that are euthanized by gas or chemical methods.

Sodium Pentobarbital 150 mg/kg IP, or CO2 asphyxiation, this will be followed by removal of the head.
Alternatively, rats will be under isoflurane anesthesia while thoracotomized and the heart removed.

/II. Animal Locations and Transport

Questions 35-39

35. Please indicate building(s) and room number(s) of research laboratories where live animals will be transported, housed or where animal procedures will be performed. Do not include vivaria or vivarium procedure rooms here.

Building:	[redacted]	Room Number:	[redacted]
Building:	[redacted]	Room Number:	[redacted]
Building:		Room Number:	

36. Transport of animals out of the animal housing facility (vivarium) is strongly discouraged and return trips to the vivarium are not only discouraged but prohibited for some animal populations. If animals must be transported out of the vivarium, indicate where animals will be taken, why animals are transported, what procedures are performed on animals in the laboratory, how animals will be transported, how often animals will be transported and whether or not animals will be returned to the vivarium. If you do not transport animals, please state N/A.

Animals will be transported by [redacted] in a micro isolation cages from the vivarium to the PET, US, and MR in the [redacted].
Animals that will exercise 24 hrs prior to diving will do so in the vivarium, i.e. no animal will be transported back to the vivarium once coming to the lab for diving/gas experiments.

I have read and agree to abide by the Policy 33 Transportation of Rodents.

37. Request for Satellite Housing. Housing animals outside of an approved ACP vivarium for greater than 12 hours is against UCSD Policy 28 . If you are requesting an exception to this policy, please indicate housing site requested and justify the need to do so. If you do not keep animals out of the vivarium for greater than 12 hours, please state N/A.

Animals to be exercised one day and imaged the next will remain in [redacted] overnight, unless we receive permission to return them to their housing in [redacted]

38. Standard housing, food and water, and enrichment is provided by ACP as described in Policy 12 - Housing and Environmental Enrichment for Laboratory Animals

Check here if you require only standard ACP husbandry.

OR describe below if you require non-standard housing (such as single-housing of social species for experimental reasons), non-standard feed or water (such as food on the cage floor, specially formulated diets, additives to drinking water, addition of sunflower seeds or other food enrichment) or non-standard enrichment. Please provide scientific justification for single-housing of social species.

39. There are specific policies and guidelines for animal work that is funded through UCSD but performed away from the UCSD campus. Please read Policy 21 Inter-Institutional Research.

Check here to indicate that you will perform all experimental work at UCSD campus locations.

If any animal work will be done at other locations, please describe in detail below. Include animal work that might be done at Contract Research Organizations (CROs) or collaborator's locations such as imaging, irradiation, housing, breeding, surgeries, euthanasia, tissue collection, etc. Include locations of custom antibody production (but do not include information for antibodies purchased "off the shelf"). Include surgery performed by the vendor prior to arrival at UCSD (e.g. ovariectomy, vasectomy, adrenalectomy, catheterizations, etc). Provide all information about the location(s) where this animal work is performed: company or institutional name, address, telephone number, PHS assurance number, and AAALAC accreditation date.

/III. Hazardous Agents

This project DOES NOT include hazardous chemicals, microbial organisms, recombinant DNA, human cells, radioactive materials, or animals that carry zoonoses. If any hazardous agents are used in live animals, please describe below. A project that uses hazardous agents may not begin until the investigator has obtained any necessary EH&S BUA, RUA or appropriate IBC approval.

Agent:

-- Other hazard not listed

Reviewer: [redacted] **Review Date:** 11/12/2019

Prescribe handling for research staff:

Specific Description of Hazard (strain, type, etc.)
Radioactive isotope of nitrogen gas. 13N.

How is Hazard used?
The positron emitting isotope 13N has a radioactivity with short time span. Half-life is 9.96minutes. The rats will breathe some of this gas instead of regular atmospheric nitrogen gas. Imaging will be done in a PET for as long as there is measurable activity (about 2-3 hours).

R4: High level radiation

- **Follow conditions of the RUA 720 at all times**
- **Follow Section 2.3 of the Radiation Safety Manual (Care and Handling of Animals Containing Radioactivity)**
- RUA holder is responsible for posting rooms, conducting routine surveys of cages and rooms, changing radioactive animal bedding, removing and disposing of all radioactive waste and carcasses, decontaminating cages and rooms and de-posting rooms
- Monthly contamination surveys for low use labs and weekly contamination survey for high use labs
- **Ensure proper labeling of all animal cages and rooms at all times**
- Do not handle unshielded sources directly
- Store behind lead shielding
- Use lead shielding to minimize exposure
- Extremity and whole body dosimetry required
- EH&S approved isotope administration, imaging and transportation procedures
- EH&S approved waste handling, storage and decay procedures
- PET specific lead shielding required
- Perform radiation exposure surveys during and contamination surveys after procedure

NOTE: wear lab coat, gloves, and safety glasses. To avoid cross contamination never wear gloves outside of the research lab

Prescribe handling for animal care staff:

- Prior to entering room an ACP employee must observe signage posted on the door (See "Typical Caution Radioactive Material" signs used at UCSD) and follow all instructions for proper PPE before entering the room.
- Put on appropriate PPE prior to entering the room as follows:

- Lab coat
- Gloves
- Safety glasses

☐ Hazard Use has **NOT** been approved by EH&S
☐ Safety Considerations Meeting is Required

Reviewer: [redacted] **Review Date:** 5/4/2020

Prescribe handling for research staff:

For the safe use of anesthetic gas in research environments use:

blink.ucsd.edu/safety/research-lab/chemical/anesthetic.html

Use the following link to report a work-related injury, illness, or hazardous material exposure

http://blink.ucsd.edu/go/injuryreport

Agent:

Isoflurane

Specific Description of Hazard (strain, type, etc.)
Isoflurane 1-3%

Prescribe handling for animal care staff:

For the safe use of anesthetic gas in research environments use:

blink.ucsd.edu/safety/research-lab/chemical/anesthetic.html

Use the following link to report a work-related injury, illness, or hazardous material exposure

http://blink.ucsd.edu/go/injuryreport

How is Hazard used?
Anesthetic

☐ Hazard Use has **NOT** been approved by EH&S
☐ Safety Considerations Meeting is Required

Reviewer: [redacted] **Review Date:** 7/18/2023

Prescribe handling for research staff:

For information about and approval to use controlled substances visit

http://blink.ucsd.edu/safety/research-lab/controlled-substances/

And follow directions

For all injuries and incidents see

http://blink.ucsd.edu/go/injuryreport

Agent:

-- Other hazard not listed

Specific Description of Hazard (strain, type, etc.)
Sodium Pentobarbital

Prescribe handling for animal care staff:

For information about and approval to use controlled substances visit

http://blink.ucsd.edu/safety/research-lab/controlled-substances/

And follow directions

For all injuries and incidents see

http://blink.ucsd.edu/go/injuryreport

How is Hazard used?
Sodium pentobarb will be used as a euthanasia agent

☐ Hazard Use has **NOT** been approved by EH&S
☐ Safety Considerations Meeting is Required

X. Funding

Identify all funding for this project

Grant PI	UCSD Proposal #:(20xx-xxxx)	Covering Dates From (mm/dd/yy)
[redacted]	EPD Proposal# is [redacted]	10/01/2021 to 12/31/23

Agency

Office of Naval Research

Grant Title

[redacted]

☐ Grant is extramural, competitive, and peer-reviewed

☑ Funds have been awarded

☐ Funds Pending

Grant PI	UCSD Proposal #:(20xx-xxxx)	Covering Dates From (mm/dd/yy)
		to

Agency

Grant Title

☐ Grant is extramural, competitive, and peer-reviewed

☐ Funds have been awarded

☐ Funds Pending

Provide additional funding information here:

Other regulatory approvals that you may need before beginning your project

☑ I have read all of the documents listed here which apply to my animal protocol

Are you using human embryonic stem cells?
You are responsible for knowing and following the federal, state, and UCSD regulations and obtaining approval from the UCSD Embryonic Stem Cell Research Oversight Committee. For more information go to the UCSD Stem Cell website.

Are you using human blood or other tissue in animals?
You are responsible for knowing federal, state and UCSD IRB regulations and may need to receive IRB approval prior to beginning your studies. Please read the information at the UCSD IRB website.

Is any of your funding from a non-federal source (including departmental funds, gifts, private grants, clinical trial agreements, lab service agreements, and for profit contracts)? Is anyone listed on your protocol an employee, officer, or stockholder of the funding source? Do you receive income from the funding source?
You are responsible for knowing and following the UCSD, state and federal regulations pertaining to Conflict of Interest. For information and application go to the Conflict of Interest website.

Will you use controlled substances as anesthetics, analgesics or test substances in this protocol?
You are responsible for knowing and following UCSD rules and you must obtain a CSUA. More information and forms at EH&S Controlled Substances Acquisition, Storage, and Disposal Requirements.

Are you using hazardous agents?
You must obtain any necessary BUA, RUA, or IBC approval before starting this project. You must train your laboratory personnel and key Animal Care Program personnel regarding safe handling, disposal and cleanup of the hazardous agent. You must assure that areas where hazardous agents are used have appropriate signage and necessary PPE available. More information and appropriate forms are available at Environment, Health and Safety website.

Will you use anesthetic gases in this protocol?
Follow the Safe Use of Anesthetic Gases in Research Environments guidelinesto control the risk of exposure to waste anesthetic gases.

Do you have employees or students that handle animals as a part of their job or training?
You must assure that each of your employees and students has submitted an online Personnel Qualifications form (PQ), that each person has been added to your protocol, and that each person has taken the Risk Assessment Questionnaire and been given the opportunity to participate in the Medical Surveillance program. For more information, go to the Occupational Health Policy website.

Are you using animals in teaching undergraduate, graduate or continuing education students?
You must educate students on the ethical use of animals in research, appropriate handling, and student health and safety concerns. You must post and/or distribute the following poster to students before exposure to animals: Student Health Flyer.

Will you use the UCSD Center for fMRI?
You are responsible for submitting an application to the fMRI Center Biomedical Applications Committee (fCBAC) at http://fmri.ucsd.edu.

Are you working with Dual Use Research Agents or performing Dual Use Research of Concern (DURC)?
You are responsible for reporting DURC agents used in research to the UCSD DURC Institutional Review Entity for DURC review through the BUA process. For more information on DURC agents, the experimental effects of concern and the DURC review and approval process go to the UCSD DURC website.

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

◊. Investigator's Assurance For the Humane Care and Use of Animals Used in Teaching and Research

☑ I am the Principal Investigator and I have thoroughly read, agree with, and will actively promote and enforce all of the assurances listed below:

1. I agree to abide by PHS Policy, USDA Regulations, UCSD policies for the care and use of animals, the provisions of the ILAR Guide to the Care and Use of Laboratory Animals, and all other federal, state, and local laws and regulations governing the use of animals in research.
2. I understand that emergency veterinary care will be administered to animals showing evidence of pain or illness, in addition to routine veterinary care as prescribed for individual species. I understand that it is my responsibility to provide current and updated emergency contact information for personnel who must be contacted in an animal emergency. I understand that any unanticipated pain or distress must be reported to the veterinarian or his/her designee.
3. I assure that I have consulted a veterinarian in the preparation of this proposal, if it includes procedures that could cause pain and distress to a vertebrate animal.
4. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified biomedical scientist listed on this protocol.
5. I certify that all personnel having direct animal contact, including myself, have been trained in humane and scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project.
6. I certify that all personnel in this project will attend Orientation to Animal Research and all mandatory classes as determined by each individuals Personnel Qualifications Form.
7. I understand that the use of hazardous or controlled materials in animals may only be initiated after authorization from the applicable Campus Safety Committees and EH&S, and used in the manner/purpose for which they are approved in compliance with Federal, State, local and UC San Diego requirements. I am responsible for complying with all safety related information (section VIII) of the protocol as well as complying with EH&S authorizations such as Biohazardous Use Authorization (BUA), Controlled Substance Use Authorization (CSUA), Radioactive Use Authorization (RUA), Laser Use Authorization (LUA), and documenting the proper use of such hazards or processes in the Hazard Control Plan(s).
8. I certify that all personnel working on this protocol will be given the opportunity to participate in the Medical Monitoring Program at the Center for Occupational and Environmental Medicine (COEM). All personnel on this protocol will be made aware of the hazards involving the use of live animals and tissues.
9. I understand that I must submit an amendment for any proposed changes to this protocol and wait for IACUC approval before beginning the work.
10. I understand that should I use the project described in this application as a basis for a proposal for funding (either extramural or intramural), it is my responsibility to ensure that the description of animal use in such funding proposals are identical in principle to that contained in this application.
11. I understand it is the responsibility of the Principal Investigator to ensure the safe and ethical conduct of all research conducted under this protocol, and to assure that all research is carried out following federal, state, local, and UCSD policies governing animal research.
12. I certify that I will maintain complete, up-to-date and accessible records of procedures on animals as required by policy and regulation.
13. I declare that the information provided in the accompanying protocol is accurate to the best of my knowledge.
14. I certify that all state, federal and international permits for the use of the animals described in this protocol are in place (or will be in place before studies begin) including those permits mandated by the Department of Commerce, Marine Mammal Protection Act, Bureau of Land Management, National Forest Service, and foreign countries.
15. I acknowledge that clicking on the "submit protocol" button below is equivalent to an electronic signature.