

BUMED Animal Use Appendix for Research Involving Animals
ABBREVIATED VERSION

*****NOTE: BUMED will ONLY review protocols that have been approved by your IACUC.*****

**** Animal work MAY NOT be initiated until the awardee receives BUMED approval.****

Animal work initiated without BUMED approval is noncompliant and may not be funded.

Institutions using DoD funds to support the use of animals in research, product development, testing and education projects must provide electronic copies of the following documents to the Department of the Navy (DON) Bureau of Medicine and Surgery (BUMED) Veterinary Affairs Office for review and approval prior to initiation:

- a. A copy of their **IACUC-approved institutional protocol(s)** (BUMED will ONLY review **approved** protocols) and documentation of IACUC approval
- b. A copy of all existing IACUC-approved protocol amendments or modifications and documentation of IACUC approval (**future modifications or amendments** must be reviewed and approved by BUMED **PRIOR** to implementation)
- c. A completed Appendix for each IACUC-approved protocol.

This requirement also applies to all subcontractors using animals in support of DoD-funded projects or programs.

Specific information requested in the following animal use Appendix is derived from requirements in the Animal Welfare Regulations (AWRs), the *Guide for the Care and Use of Laboratory Animals*, and other applicable Federal and DoD regulations. The DoD policies and requirements for the use of animals in DoD-supported research, development, testing and evaluation are described in DoD Instruction 3216.01, dated September 13, 2010 and SECNAVINST 3900.38C, *The Care and Use of Laboratory Animals in DOD Programs*, dated February 16, 2005. These requirements differ from those of other funding agencies. Use of the Appendix is intended to meet the requirements of these documents.

Questions concerning animal use and review should be directed to BUMED Veterinary Affairs:


Phone: 301-619-9241 or 301-619-9224

Email: DON – VRPP, usn.ncr.bumedfchva.mbx.don---vrpp@mail.mil

Mail: Department of the Navy Bureau of Medicine and Surgery
ATTN: Director for Veterinary Affairs
Bldg 1564 Room 122
1564 Freedman Drive
Fort Detrick, MD 21702

Each section of this Appendix must be completed. To assist you in completing this appendix, instructions and explanations are provided as *hidden, red and italicized text*. To view the instructions and/or examples for each section, select the “**Show/Hide ¶**” button on your tool bar (the button itself appears as the ¶ symbol). To print the hidden text, select “Print Hidden Text” in the print options section. It is important that you carefully **read the instructions** for each paragraph to ensure you provide a comprehensive response. Begin typing responses after the colon (“:”) for each section to ensure your typing is not within the hidden text. Submit electronic copies of the appendix only; please do not submit printed copies to BUMED. Any section of the Appendix that is not applicable to your proposal, e.g., no surgery or no prolonged restraint, should be marked “**No**” or “**N/A**”. There are no space limitations for the responses.

It is essential that only animal studies or procedures documented in an IACUC–approved protocol or amendment be performed at your facility. BUMED will collect animal usage information for end-of-year DoD animal use reports separate from the grant annual progress reporting requirement throughout the life of your award. For this reason, Principal Investigators or other delegated research personnel should keep accurate records and be able to provide an audit trail of all animal use that correlates to their approved protocol.

Yes No All animal studies described in the attached IACUC-approved protocol are funded by this DOD award/contract. If no,  ! You must use the **full version** of the Animal Use Appendix.

1. Administrative Data: (Provide Attending Vet, IACUC, and Research Office info for the work site.)

DoD/Contract PI Name: Jay B. Dean, PhD

Grant/Contract PI Email: jaydean@usf.edu **Phone:** 813-974-1547

Protocol PI Name: Jay B. Dean, PhD

Protocol PI Email: jaydean@usf.edu **Phone:** 813-974-1547

Animal Research Site (RS): University of South Florida, Tampa, FL

RS Attending Veterinarian: Dr. Norman Wiltshire, DVM, DACLAM, Assoc. Dir. Comp. Med.

Attending Vet Email: wiltshire@usf.edu **Phone:** (813) 974-3673

RS IACUC Point of Contact (POC): Dr. Shari Pilon-Thomas (Vice-Chair USF IACUC);
[Note: Dr. Jay B. Dean (P.I.) is the Chair of the USF IACUC]

IACUC POC Email: Shari.Pilon-Thomas@moffitt.org **Phone:** (813) 745-4247

RS Grants Office Point of Contact (POC): Ms. Bethany Kincaid

Grants Office POC Email: bkincaid@usf.edu **Phone:** (813) 974-5044

Animal Protocol Title: Mitigation of CNS oxygen toxicity: mitochondria-targeted antioxidant therapy and aquaporin gas channel

2. Total Number of Animals Used (by Species) and USDA Pain/Distress Category:

SPECIES	HIGHEST USDA PAIN/DISTRESS CATEGORY (B, C, D, E*) See table below for definitions	TOTAL NUMBER
Sprague-Dawley rat	B	25
Sprague-Dawley rat	C	122
Sprague-Dawley rat	E	153
Mouse: Aquaporin-4 KO	E	20
Mouse: C57BL/6 WT	E	50

USDA Pain/Distress Category Definitions:

- Column B:** *Animals being bred, and animals being held for use in research, testing, teaching, experiments or surgery but not yet used for those purposes.*
- Column C:** *List the number of animals that will experience no more than slight or momentary pain or distress as a result of experimental manipulations or procedures on this protocol.*

- **Column D:** List the number of animals that will potentially experience more than momentary or slight pain or distress that **WILL** be alleviated through the use of anesthetics and/or analgesics.
- **Column E:** List the number of animals that will experience more than momentary or slight pain or distress that **WILL NOT** be alleviated or relieved with anesthetics or analgesics. **If any animals are listed in USDA Column E (Unalleviated Pain or Distress), the PI must provide a scientifically valid justification for withholding pain relieving medication:**
 - USDA Pain Category E (= pain category C in USF IACUC animal use protocol): Animals listed in USDA Pain Category E will be exposed to hyperbaric oxygen until onset of two bouts of seizures, which may cause discomfort. The potential for discomfort is why these animals are placed in USDA pain category E. The animals are unanesthetized in these experiments so that their visible behaviors can be used as one of the criteria to score onset of a seizure; we use a six-point scale of behavior to evaluate the type of seizures. Sensitivity to hyperbaric oxygen is blunted in an anesthetized animal and thus the latency time to seizures is severely lengthened plus a higher oxygen pressure is required. After the second bout of seizures occurs the breathing atmosphere is changed from 100% O₂ to air and the animal chamber is decompressed at a rate of ~1 atmosphere/minute. The 1st bout of seizures is short lasting (seconds) and during the interictal period before the 2nd bout of seizures the animal, remarkably, appears fine for all intents and purposes based on its behavior. After recovery at room pressure, the animal appears normal based on its behavior.

3. Literature Search for Unnecessary Duplication: This search is required for all animal use proposals. Note the DoD-specific database requirements in subparagraph a.

a. Literature Source(s) Searched:

<http://www.ntis.gov/products/fedrip.aspx> <http://projectreporter.nih.gov/reporter.cfm>
Federal Research in Progress (FEDRIP) <http://www.ntis.gov/products/fedrip.aspx>

or

Research Portfolio Online Reporting Tool Expenditures and Results (RePORTER)
<http://projectreporter.nih.gov/reporter.cfm>

b. Date of Search: 16-17 December 2019

c. Years Covered By Search: 1994-2019

d. Key Words and/or Search Strategy:

Search 1: ((mitochondria targeted antioxidant therapy) or MitoQ or Mitoquinone), hyperbaric oxygen, oxygen toxicity, rat, ((brain slice) or (radio telemetry))

Search 2: hyperbaric oxygen, oxygen toxicity, seizure, aquaporin, gas channel, mouse

e. Results of Search:

Search 1: Searched in Projects, Publications and publication year limited between 1994 and 2019, Limit to: Project Abstracts, Admin IC: All, Fiscal Year: All Fiscal Years. Your search resulted in 0 projects. There were no relevant projects and publications found, even with altering the combination of words used in a search.

Search 2: Text Search: Search in Projects, Publications and publication year limited between 1994 and 2019, Limit to: Project Abstracts, Admin IC: All, Fiscal Year: Active Projects. Your search resulted in 0 projects. There were no relevant projects and publications found, even with altering the combination of words used in a search.

******Questions 4-10 refer to the Research Site and Protocol Principal Investigator.******

Information and/or documents required in questions 4-8 should be obtained from the research site’s IACUC or veterinary staff. Documents may be provided directly to BUMED by the Protocol Principal Investigator (PI) or institution staffs. If the Protocol PI prefers that BUMED staff contact the institution to obtain this information and/or documents, the PI must specifically request this action in writing. The Protocol PI should contact BUMED (see contact information on cover page) to arrange for submission of this written request.

4. Institutional Animal Care and Use Committee(s) (IACUC) Approval(s):

Institutional Animal Use Protocol Number: IS00007438

IACUC (initial) approval date: December 13, 2019 Protocol expiration (rewrite) date: December 10, 2022

5. Veterinary Care: Provide a brief description of the veterinary care plan at the research site. Describe routine care; weekend, holiday, and emergency care; and identify whether the attending veterinarian is on staff full-time or by contract.

USF MCOM Comparative Medicine is fully accredited by AAALAC International as program #000434, is managed in accordance with the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Regulations, the PHS Policy, the FDA Good Laboratory Practices, and the IACUC Principles and Procedures of Animal Care and Use, has an assurance #D-16-00589 (A4100-01) on file with OLAW/PHS and maintains registration #58-R-0015 with USDA/APHIS/AC.

Comparative Medicine veterinarians are full-time staff, Florida-licensed, members of the AVMA, and are specialty board certified by ACLAM, and/or have research doctorates. Comparative Medicine maintains institutional membership with AALAS. All program staff are AALAS-certified, and many are either registered with the Society of Quality Assurance, and/or certified by the Academy of Surgical Research or are State of Florida-certified veterinary technicians.

Daily routine, weekend, holiday, and emergency animal care is provided by animal care program staff, all of whom are certified by the American Association for Laboratory Animal Science (AALAS), and more staff are certified at the highest, technologist level {LATG} than any other level. Cage changes occur twice weekly. Health surveillance occurs twice daily and assesses: food and water intake, fecal output, assurance of water supply and patency, and general appearance of animals (e.g. presence of lesions/injuries, coat condition, disposition). If at any time there is a medical emergency, the clinical veterinarian is notified immediately.

6. Institutional Accreditation / Assurances:

a. Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) Accreditation (do **NOT** provide AAALAC correspondence):

Yes No Animal work is being performed at an AAALAC International-accredited facility.

b. Public Health Service Animal Welfare Assurance Statement:

Yes No Animal work is being performed at a PHS-assured facility.

c. Non-accredited, Unassured Facilities: If neither 6.a. nor 6.b. above apply to the facility where animal work is being performed, submit a statement signed by the Institutional Official that states the care and use of animals will be conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* and applicable Federal and DoD regulations.

7. Animal Procurement:

Yes No If the protocol involves Animal Welfare Act-regulated species, are the animals obtained legally from suppliers licensed by the USDA? If the supplier claims exemption from USDA licensing, provide confirmation from the research site's IACUC that the exemption criteria have been met. If work is conducted outside the US, have animals been obtained legally in accordance with national policy? If wildlife are used, provide IACUC assurance that animals have been obtained legally and provide copies of applicable state, federal and/or international capture and use permits.

8. Overseas / Foreign Country Animal Work:

Yes No Animal work will be performed outside the United States.

If "Yes," answer the following questions.

- a. What is the law or regulation governing the use of animals in research in the research site's host country? Please provide a copy or link to this law or regulation in English.
- b. Does the research site's host country adhere to European Union (EU) Directive 86/609 or EU Directive 2010/63 standards of animal housing and care?
- c. If the research site is in Canada, does the institution hold a Canadian Council on Animal Care (CCAC) certificate?
- d. Does the research site adhere to any national or international standards of animal housing and care that are more stringent than the host country's laws or regulations (such as AAALAC or CCAC)? If so, please describe below or provide a document, in English, that describes these standards.
- e. Does the research site's host country or local institute require a local ethical committee review or Animal Care and Use Committee review? If so, please describe below or provide a document, in English, describing the committee's membership, purpose, authority and function.

9. Site Visits

Yes

No

Does animal work involve at least one of the following species: dogs, cats, nonhuman primates, marine mammals? If yes, provide a planned start date for work in these species and point of contact for site visit coordination. Based on accreditation status, species used, and type of research conducted, a site visit to the performance site may be required.

10. Protocol Principal Investigator Assurances:

The law specifically requires several written assurances from the P.I. Please read and sign the assurances as indicated (this page may be photocopied and signed).

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Painful Procedures: I assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic, and/or tranquilizing drugs will be used where indicated and appropriate to minimize pain and/or distress to animals.

B. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC and the DoD Department of the Navy **Bureau of Medicine and Surgery** (BUMED) Veterinary Affairs Office prior to its implementation.

C. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

D. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to implement animal use alternatives where feasible, and conduct humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

Jay B. Dean

(Protocol Principal Investigator Printed Name)

(b) (6)

(Protocol Principal Investigator Signature and Date)

NOTE: In accordance with SECNAVINST 3900.38C, the DON BUMED Veterinary Affairs Officer (or designee thereof) will conduct a site visit to all sites using nonhuman primates, dogs, cats or marine mammals in the proposal, or where a site visit is deemed warranted.