



DEPARTMENT OF THE NAVY  
NAVAL MEDICAL RESEARCH CENTER DETACHMENT

LIMA, PERU  
UNIT NUMBER 3800  
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IN REPLY REFER TO  
NMRCINST 3900.2A  
February 27, 2004

NMRCD INSTRUCTION 3900.2A

From: Officer-in-Charge, Naval Medical Research Center Detachment  
To: All NMRCD Personnel

Subj: NMRCD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

- Ref:
- (a) 7 USC 2131-2156, Animal Welfare Act, as amended.
  - (b) SECNAVINST 3900.38.B, "The Use of Animals in DOD Programs," Jun 1984.
  - (c) Chapter 9, Code of Federal Regulation, Parts 1-3.
  - (d) BUMEDINST 3900.8. Written Animal Use Proposals.
  - (e) NMRCINST 3900.1I. Use of Animals in Medical Research.
  - (f) NIH Guide for the Care and Use of Laboratory Animals.
  - (g) Report of the AVMA Panel on Euthanasia, JAVMA 2000; 202(2): 229-249
  - (h) DOD Directive 3216.1, 17 April 1995.
  - (i) 1994 Report to the House Armed Services Committee and the House of Representatives National Security Committee entitled "Department of Defense Animal Care and Use Program".
  - (j) The United States Public Health Service Policy on the Care and Use of Laboratory Animal, 2000. Office Laboratory Animal Welfare (OLAW), National Institute of Health, Bethesda, MD 20892.

- Encl: (1) Animal Care and Use Protocol Template  
(2) Annual Protocol Review Form  
(3) USDA Pain Justification Memorandum

1. Purpose. To establish policies and assign responsibility for the protection of laboratory animals utilized in research at NMRCD. The Institutional Animal Care and Use Committee (IACUC) is appointed to ensure compliance with all regulations regarding the care and use of laboratory animals. This instruction also provides minimum training requirements for Institutional Animal Care and Use Committee (IACUC) members, and investigators or technicians who will be working with animals or animal tissues.

2. Cancellation. NMRCD Instruction 3900.2

3. Background. References (a) to (j) are guidelines set forth for the humane care and maintenance of laboratory animals. The Institutional Care and Use Committee (IACUC) is appointed to ensure compliance with all regulations regarding the care and use of laboratory animals.

4. Applicability. This instruction applies to all NMRCD investigators and collaborators utilizing laboratory animals for investigational purposes, NMRCD veterinary support staff, or personnel using NMRCD animal facilities, including any satellite facilities. Specifically, all procedures and protocols performed at NMRCD which use animals for:

- a. Research and development, testing, or evaluation (RDT&E)
- b. Clinical Investigation
- c. Diagnostic purposes
- d. Instructional programs or exhibitions

5. Definitions.

a. AAALAC - Association for the Assessment and Accreditation of Laboratory Animal Care (International). Voluntary accrediting body for demonstrating achievement of certain standards for an animal care and use program. DOD Directive mandates that all DOD research institutions shall be AAALAC accredited.

b. Alternatives - Activities that reduce, refine, or replace the use of animals in medical research as defined in reference (h).

c. Animal - Any live or dead warm-blooded vertebrate animal used for research, development, test and evaluation (RDT&E), clinical investigation, diagnostic procedures, instruction or exhibition.

d. Animal Care and Use Program - All activities involving or including animal use, veterinary services, animal facilities, animal husbandry support personnel, and/or the IACUC.

e. FIPR - Facility Inspection and Program Review. Federal law requires the Institutional Animal Care and Use Committee to inspect all animal facilities and study areas at least once every six months, and to insure the Institute's animal care and

use program is in compliance with the regulations and standards promulgated under the Animal Welfare Act and DOD regulations.

f. IACUC - Institutional Animal Care and Use Committee (IACUC). The IACUC will be established as required in reference (a). The Committee will evaluate care, treatment, housing, and use of animals. The IACUC will also certify to the Officer-in-Charge that the research facility and all procedures are in compliance with the Animal Welfare Act. The NMRCD Institutional Animal Care and Use Committee is required by law to review all proposed animal use protocols, all animal programs, and all NMRCD animal holding and animal use facilities.

g. Institutional Official - The person with ultimate responsibility concerning the animal care and use program. At NMRCD, this person is the Officer-in-Charge.

h. OLAW - Office of Laboratory Animal Welfare. The office oversees compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The office is part of the National Institute of Health, Bethesda, MD.

i. Principal Investigator (PI) - The person(s) responsible for conducting the research or teaching described in the proposed animal use protocol. The PI signs the Assurance Statement and assumes responsibility for the use and care of animals in conformance with procedures set forth in the proposed protocol and in compliance with regulation and law.

j. Protocol - A document prepared by the PI detailing the proposed use of animals. This protocol must contain specific information and be prepared according to the template provided as enclosure (1) concerning the use and care of animals, the number and type of animals required, the training of the investigators, the search for alternatives to the use of animals, justification for any pain or distress caused to the animals, information on bio-safety/biohazard conditions, cited searches of appropriate databases to ensure that the research is not unnecessarily duplicative, a discussion of the need for this research, a review of the pertinent literature, a testable scientific hypothesis, detailed experimental design addressing the hypothesis and minimization of animal usage, data analysis plan and bibliography. Once approved by the IACUC, the protocol

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becomes a "contract" between the PI and the Institutional Official.

k. Research. A systematic investigation designed to develop or contribute to general scientific knowledge, to include any project, task, test, and experiment involving the use of animals.

l. Quorum. A majority of the IACUC Committee members.

6. Policy. Specific guidelines are set forth by the Animal Welfare Act, 99-198, 1965 (as amended), reference (a), to utilize animals for research or training.

a. The facility will provide adequate living conditions, veterinary medical care, emergency veterinary medical care, and humane treatment.

b. All animals will receive analgesics, anesthetics, and sedatives unless withholding such agents is justified by the investigator and approved by the Committee.

c. The study must contribute to human or animal benefit and have reasonable prospects of yielding results, which cannot be obtained by an alternate method of study.

d. The number of animals will be kept to the minimum necessary to achieve significant results. Thorough literature review must be conducted to ensure appropriate species, no unnecessary duplication of research effort, and no alternative method.

e. Staff personnel, investigators, and each member of the research team shall be qualified to perform their functions. The facility will meet this requirement by providing training to staff, investigators, scientists, and other personnel involved with animal care and use.

f. All treatments or surgical procedures to be performed must utilize aseptic facilities and procedures. An animal will not be used in more than one major operative procedure, from which it will recover, unless there is prior approval by the Committee.

7. Authority. The IACUC, appointed by the IO, has the authority, mandated by Federal law, to act on behalf of the Institutional Official to:

a. Be directly responsible to the Officer-in-Charge for subject matter within their cognizance as outlined in this SOP and references (a) to (j). It is not the responsibility of the IACUC to prescribe methods or set standards for the design, performance or conduct of research by the staff or the research investigators.

b. Review protocols for humane care, appropriate species, number of animals, appropriate procedures (anesthesia, postoperative care, etc.), scientific validity, qualified researchers/staff, and redundancy.

c. Recommend one of the following actions to the Officer-in-Charge, based on Committee review:

Approve the protocol as submitted

(2) Approve the protocol, pending submission of identified modification.

Approve as a pilot study.

(4) Disapprove the protocol, identifying the reason(s) for such action.

d. Conduct a semiannual walk-thru of the facility and review of the animal care program. All protocols to be reviewed by the Committee must contain the information in enclosure (1).

e. Notification: Provide, in writing, the decisions of the Committee to the Officer-in-Charge and the principal Investigator. This will include approval of protocols, disapproval (with justification) of protocols, policies set or inspection results.

f. Investigate any concerns relating to laboratory animal care and use practice and suspend any activity, which violates federal laws, regulations and guidelines, DOD Regulation and/or IACUC policy.

g. Humanely euthanize an animal that is suffering from pain or distress that cannot be alleviated. Certain exceptions may apply when thorough justification is provided in approved, specific protocols.

## 8. Responsibilities.

a. The OIC, NMRC, as IO, will:

(1) Ensure that Research Development Testing and Evaluation (RDT&E), clinical investigations, diagnostic procedures, and instructional programs are conducted in compliance with applicable laws, regulations, guidelines and standards. Contract and grant programs and collaborative research facilities must also conform. If there is a conflict or difference between the regulations with respect to standards of humane care and use, then the stricter of the standards will apply.

(2) Ensure that local animal care, use, procurement, and transportation policies comply with applicable laws, regulations, guidelines, and standards.

(3) Ensure that animals will experience no unnecessary pain, suffering, or distress and that their use meets valid DOD requirements.

(4) Ensure that efforts will be made to seek and utilize procedures, which minimize pain and distress.

(5) Ensure that alternatives to the use of animals be used if they produce scientifically satisfactory results.

(6) Ensure every effort is made to reduce the number of animals used in research, that appropriate controls will be in place, and that unnecessarily duplicative research does not occur.

(7) Ensure that dogs, cats or nonhuman primates will not be used to develop offensive nuclear, biological or chemical weapon capabilities.

(8) Ensure that an Occupational Health and Safety Program constitutes part of the overall Animal Care and Use Program.

(9) Establish and appoint members to the IACUC  
appoint the Chairperson.

(10) Ensure that the IACUC Chairperson and the Attending  
Veterinarian have direct access to, and report directly to, the  
Institutional Official.

11 Review the minutes and reports submitted by  
IACUC

(12) Render reports on animal care and use programs as  
required by DOD Directive, USPHS Policy, AAALAC or the Animal  
Welfare Act.

b. Facility Veterinarian will:

(1) Ensure that all animal research is performed under  
approved protocols

(2) Review all protocols submitted by PIs for mission  
relevance, scientific merit, and program need

(a) The primary responsibility for scientific review  
belongs to the Program Director. Their signature on the  
protocol title page certifies that the protocol is  
scientifically meritorious and relevant to the Program's  
mission.

(b) In the event that the Program Director is also  
an investigator on the protocol, then a statement detailing how  
scientific review was performed must be included in the  
protocol.

(3) Ensure that all studies are conducted under current  
occupational health and safety as well as bio-safety guidelines.

(4) Will maintain records for the following:

(a) A list of any offspring born of any animal while  
in NMRCD's possession or under its control.

(b) The name and address of the person from whom the  
dog, cat, or nonhuman primate was purchased or otherwise

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acquired, whether or not the person is required to be licensed or registered under the Animal Welfare Act.

(c) The USDA license or registration number of the person if he/she is licensed or registered under the Animal Welfare Act;

(d) The date of acquisition of each dog, cat, or nonhuman primate;

(e) The official USDA tag number or tattoo assigned to each dog or cat;

(f) A description of each dog, cat, or nonhuman primate, which shall include:

- 1) Species and breed or type of animal;
- 2) Gender;
- 3) Date of birth or approximate age; and
- 4) Color and any distinctive markings

(g) Any identification number or mark assigned to each dog, cat, or nonhuman primate by the Institute.

(5) Prior to transferring ownership of any live dog, cat, or non-human primate to another facility or individual, the Animal Facility Department will make and maintain records, which fully and correctly disclose the following information:

(a) The name and address of the person to whom a live dog, cat, or non-human primate is transported, sold, or otherwise disposed;

(b) The date of transportation, sale, euthanasia, or other disposition of the animal;

(c) The method of transportation including the name of the initial carrier or intermediate handler, or if a privately owned vehicle is used to transport the animal, the name of the owner of the privately owned vehicle.

g. All records and reports shall be maintained for at least three years.

c. The IACUC will:

(1) Review and approve all proposed animal use protocols to be conducted by PIs for compliance with applicable laws, regulations, guidelines and standards.

(2) Ensure that an annual report is submitted for each protocol.

(3) Semiannually review, inspect and approve

(a) The animal care and use program for compliance with all applicable laws, regulations, guidelines and standards as well as IACUC policies and SOPs.

(b) All animal facilities, including laboratories where animal work is performed and/or animals are housed.

(4) Prepare a report of deficiencies found, a plan for correction of the deficiencies found, and the facility's adherence to Federal law. The Facility Inspection and Program Review (FIPR) report will be prepared IAW DOD Directive 3216.1.

(5) Review and investigate concerns or complaints involving the use of animals. No person shall be discriminated against or suffer reprisals for reporting violations.

(6) Suspend any activity, which violates applicable laws, regulations, or Detachment policies, or any use of animals that is conducted without an approved protocol or approved modification to a protocol.

(7) Make recommendations to the Institutional Official regarding the animal care and use program

(8) Ensure that all scientists, technicians and other personnel involved in the animal care and use program are qualified to perform their duties. Qualifications are described in the protocol and training is recorded in the IACUC office.

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(9) Ensure that scientists, technicians and other personnel are provided with training opportunities to enhance their knowledge of principles of humane animal care and use.

(10) Ensure AAALAC accreditation of the Detachment and the animal care and use program.

File annual reports required by AAALAC, OLAW, USDA and DOD.

d The Chair, IACUC will:

(1) Call and conduct meetings of the IACUC as necessary in accordance with references (a) through (j).

(2) Ensure that all animal use protocols are reviewed and approved by the IACUC in accordance with applicable laws, regulations, guidelines and standards.

(3) Ensure that the composition of the IACUC meets the requirements of all applicable laws, regulations, guidelines and standards.

(4) Call and conduct semi-annual (every six months) reviews of the animal care and use program.

(5) Keep a current list of facility deficiencies and update it semi-annually. Prepare a plan and time schedule for correction of facility deficiencies.

Direct recording, preparation and maintenance of the minutes of IACUC meetings and submit them to the Institutional Official.

(7) Direct maintenance of files of approved protocols, inspections, training and correspondence pertaining to the activities of the IACUC.

(8) Request that the PI attend the IACUC meeting where his/her protocol (or a related animal care and use issue) is to be discussed.

(9) Notify the PI in writing when the protocol is approved by the IACUC and when the PI may commence his/her use of animals as described in the approved protocol.

(10) Investigate, or appoint a subcommittee to investigate any reports of inhumane or inappropriate use of research animals, or violation of policy.

(11) Delegate administrative functions as necessary to the IACUC Administrator.

e. The Principal Investigator (PI) will:

(1) Prepare, submit and have an approved protocol for the use of animals before any research using animals is performed. The specifics of how to prepare an animal protocol are described in this instruction and in the standard DOD Protocol Template, provided as Enclosure (1).

(2) Obtain approval and signature by the protocol consulting veterinarian, statistician, and supporting program personnel prior to submission of protocols to the IACUC. Review by program officials and their subsequent signatures serve to validate scientific mission relevance.

(3) Sign Assurance Statement and assume responsibility for the performance of the research conducted under the protocol, and for assuring the continued humane care and use of animals used in the research for which he/she is PI. Furthermore:

(a) The research must be performed in accordance with the description provided in the IACUC-approved protocol and approved major modifications.

(b) Adherence to approved protocols, attention to animal health and to unexpected changes in animal facilities, safety procedures, and strict oversight of technicians working on the protocol are the responsibility of each PI.

(c) The PI must ensure that he/she and all personnel working on the protocol will be properly trained in basic animal care and use principles.

(d) Each PI is charged to keep abreast of new techniques that could reduce the number of animals needed, could reduce pain or distress, or could replace animals with non-animal systems.

(4) Obtain approval for modifications to an approved protocol before modifications are implemented. There are two categories of modifications (or amendments):

(a) *Major modifications* include the following:

- i. Changing the scientific direction of a protocol
- ii. Increasing the number of non-human primates, dogs or cats by any percentage, or increasing the number of small animals by more than 10%;
- iii. Changing the species of the animals approved in the original protocol;
- iv. Changing the USDA pain code of the protocol to a more severe rating;
- v. Increasing the biohazards on the protocol;
- vi. Adding surgery to the protocol;
- vii. Adding multiple minor modifications;
- viii. Changing the Principal Investigator. *The new PI must submit a training statement with his/her request, and a signed assurance statement.*

(b) *Minor modifications* to the original protocol do not require full IACUC approval, but must be submitted to the IACUC Chair. Guidance from IACUC members or the consulting veterinarian should be sought when the PI is unsure whether proposed changes constitute a major or minor modification. Typical minor modifications might include the following:

- i Changing doses or routes of administration of a drug

ii. Recording or measuring additional variables (e.g. locomotor activity, heart rate) in the whole animal that are no more invasive than procedures currently approved under the protocol and that do not increase the Pain Category of the protocol;

iii. Using already collected blood or tissue from an animal to measure additional variables not specified in the original protocol;

iv. Adding additional investigative compounds or veterinary health drugs that are functionally similar to one another;

v. Changing small animal numbers by less than 10% of the original number.

(c) All modifications must be documented in a memorandum and provided to the Program Director, IACUC representative, and the protocol's consulting veterinarian provided to the Chair, IACUC for filing with the protocol record. All modifications should be noted in the annual review report of the protocol.

(d) Major modifications must be submitted and approved as a protocol amendment and processed through the approval process required of the original protocol.

(e) Memorandums for both minor modifications and major modifications must state the proposed modification, justification for the modification, personnel training documentation (when applicable) and an assurance statement that the changes are in compliance with all animal welfare regulations and guidelines. Memoranda should include a brief commentary of the progress of the protocol thus far.

(5) Ensure that all individuals, and all activities associated with any protocol involving biohazards are in compliance with current CDC/NIH, DOD guidelines and regulations, as well as local SOPs.

(6) Assume responsibility for the disposition of animals following their use on the protocol by coordinating with the Animal Facility Department to determine whether euthanasia,

return of the animals to the colony or to another protocol is the best course of action.

(7) Submit annual Protocol Review Reports to the IACUC as requested, describing progress, conclusions and publications as well as any developments in the field that might alter the number of animals needed or the procedures to be used. Protocols are generally reviewed and approved for a 3-year period, but annual reports to the IACUC by the PI are reviewed to determine whether each protocol should continue. Modifications made in the protocol during the year should be detailed in the annual report.

(8) Submit, if required, information requested by the IACUC as a result of their Facility Inspection and Program Review, and address any deficiencies in the PI's area of responsibility cited by the FIPR.

(9) Wait until official written approval is received from the Chair, IACUC authorizing that the protocol may be initiated, before beginning research.

(10) Investigators wishing to conduct research involving animal by-products (tissue, blood, cells, antibodies, biochemical's, etc.) obtained either from commercial sources, a specific contract or other source may do so without IACUC approval. However, transport or planned use of animal by-products that have the potential to carry animal pathogens into the animal facility must be brought to the attention of the Attending Veterinarian and the Chair, IACUC.

f The Animal Facility Department will

(1) Procure, maintain and provide health care to animals housed in Detachment facilities and maintain required records of those activities. Provision of animal husbandry and health care, animal facility maintenance and other Animal Facility Department responsibilities shall be conducted in accordance with applicable laws, regulations, guidelines and standards, including all extant Animal Facility Department Standard Operating Procedures.

(2) Provide protocol support as needed to research personnel to ensure that all animal use procedures are performed

in accordance with applicable laws, regulations standards and guidelines.

(3) Provide training to investigators and technicians with regard to animal care and use.

(4) Provide veterinary consultation to Principal Investigators during the draft stages of each protocol

5) Provide administrative support for the IACUC

(6) Assist the IACUC in maintaining the Detachment's AAALAC Accreditation status and related documentation.

(7) The Animal Facility Department will maintain records which fully and correctly disclose information concerning each live dog, cat, or nonhuman primate purchased or otherwise acquired, owned, held, or otherwise in their possession or under Detachment control, transported, euthanized, sold, or otherwise disposed of by the Detachment.

#### 9. IACUC Membership.

a. The IACUC membership may vary with the needs of the Detachment, but be in accordance with the 1985 amendments to the Animal Welfare Act, USPHS Policy, DOD Directive 3216.1, and SECNAVINST3900.38B. The voting membership will include, but not be limited to:

(1) A senior scientist experienced in animal research;

(2) A veterinarian trained in laboratory animal science and preferably, with experience in research animal care and use that has direct program responsibility for activities involving animals at the Detachment;

(3) At least one person representing community interests (this individual cannot have any liaison with the Detachment other than as a member of the IACUC and shall not be a member of the immediate family of a person affiliated with the Detachment);

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(4) At least one person whose primary background is outside of biomedical science;

(5) A representative from each Program performing animal research;

6 A biostatistician; (if Applicable)

(7) A person responsible for safety, bio-safety and occupational health issues;

(8) The recorder of the minutes, a non-voting member who will be the IACUC Administrator;

(9) The Chair, IACUC, to be appointed by the Institutional Official;

(10) All members shall have alternate members who will be trained in IACUC procedures and responsibilities, will receive the same general information as IACUC members to stay abreast of current issues, and who may serve on various IACUC subcommittees;

(11) There shall be no more than one voting member from any single Department, unless one member is acting in another capacity separate from representation to that Department;

(12) Nonvoting or Ad Hoc members include the IACUC Administrator, consulting veterinarians, animal care personnel, technicians, and other consultants, otherwise appointed by the OIC and Chair, IACUC;

b. Should expertise not be available in-house, a subject matter expert may be called to advise the Committee.

c. Nonaffiliated members must obtain eight hours of training in laboratory animal care issues, as directed by DOD Directive 3216.1.

d. Each IACUC Department representative (or alternate) shall ensure that all protocols coming from their respective Departments are in order. This member shall sign the protocol signifying that the protocol is ready for IACUC review.

10. IACUC PROCEDURES:

a. Institutional Animal Care and Use Committee Meetings and other responsibilities:

(1) The IACUC will meet at least quarterly intervals or as deemed necessary by the IACUC Chair to review and discuss protocols, reports, the results of program and facility reviews, and other animal care and use issues brought before the Committee.

(2) A quorum consisting of a majority of the IACUC voting membership is required for each official meeting. No member may vote on a protocol for which that member has a conflict of interest (i.e. is a co-investigator) and that member may not be counted toward a quorum for that vote.

(3) Prior to the IACUC meeting, members will receive packets or email attachments containing materials to support the business conducted at the meetings. The packets or email attachments may include copies of protocols to be reviewed, minutes of previous meetings, a list of protocols approved by the IACUC during the interim since the last meeting, inspection reports, and general information pertaining to facilities and the general health and welfare of research animals.

(4) IACUC members should contact the Chair or the IACUC Administrator 10 days prior to a regularly scheduled meeting to place issues on the agenda, or to request that a consultant, investigator, or other personnel be invited to the meeting.

A review and inspection of the Animal Care and Use Program and facilities will be made by the IACUC every six months.

(6) Subcommittees of the IACUC will consist of at least **three** IACUC members or alternates

(7) Reports of the IACUC on evaluations and inspections will be reviewed and signed by a majority of IACUC members. Minority views, if expressed, must be included. These reports are to be forwarded to the IO within ten working days of the evaluation.

(8) Each year, IACUC members will review the annual Protocol Reports submitted for each active protocol.

b. The IACUC will establish and publicize procedures for reporting conditions or procedures that any observer feels violate humane care and use of animals, and violates the Animal Care and Use Program.

(1) All allegations reported to the IACUC will be evaluated by the IACUC Chair, Attending Veterinarian and the IACUC Administrator, and reported to the IO. This subcommittee will make a determination if there is any basis for the allegation. All written allegations, and allegations determined to have basis, will be investigated.

(2) The IACUC Chair will designate a sub-Committee to investigate the complaint. The results of all completed investigations will be reviewed by the IACUC, and submitted to the IO. The IACUC Chair or subcommittee can request the full IACUC be convened to discuss the complaint at any time.

(3) All persons involved will be informed of the purpose of the investigation and the manner in which it will be conducted.

(4) Those against whom the complaint is addressed will have an opportunity to explain their side of the issue.

(5) The results of the IACUC investigation will be available to all involved, and immediately provided to the IO

(6) The IACUC may suspend a previously approved protocol according to section 2.31, paragraph c.6. of the Animal Welfare Act. In brief, the IACUC may suspend a protocol only after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present.

c Protocol Review

(1) All animal use protocols, after receiving review and signature from the consulting veterinarian, a statistician, the Department Chief, any additional support personnel, and the IACUC member, shall be sent to the IACUC Administrator for IACUC

review. The IACUC Administrator will submit one copy of the protocol to the IACUC Chair prior to submission to the IACUC.

(2) Each protocol to be reviewed will be submitted to each voting IACUC member. The members will have a time period of seven working days to review, comment on, and make a decision about how the protocol will be processed.

(3) Instead of voting or acting directly on the protocol, the IACUC Chair and IACUC Administrator may decide whether the protocol can be handled by the Chair or requires discussion at a full IACUC meeting. (If Applicable)

(4) Each member of the IACUC may contact the PI directly, or ask the IACUC administrator to do so, to ask any questions about the protocol. If disagreements between an IACUC member and the PI cannot be reconciled, then the issues should be brought before a meeting of the full IACUC's review and vote.

(5) The protocol may be approved (by majority vote) as is or pending revisions. The Committee may authorize the Chair to approve the revised protocol when submitted or the IACUC may require the revised protocol to be resubmitted to the next full meeting for approval. The protocol is officially approved and research can begin when the PI receives official written notification from the Chair, IACUC.

(6) The IACUC administrator will communicate the IACUC's comments and requests to the PI, and it is the responsibility of the PI to assure that all revisions requested by IACUC members are addressed and incorporated into the protocol before the protocol is forwarded to the IACUC Chair for approval.

(7) In reviewing the protocol or protocol amendment, the IACUC will consider whether the protocol contains all required management and regulatory elements as defined by SECNAVINST 3900.38.B and the Standard DOD Protocol Template (Enclosure 1), and whether the body of the protocol is understandable and complete. The IACUC will determine if the proposal meets the current standards and practices for pain management, adjuvant use, end-point determination, etc., as defined in applicable laws, regulations, guidelines and standards.

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(8) The IACUC will evaluate whether proposed protocols involving cats, dogs, or nonhuman primates can only be performed in those species or whether some other species or test system could produce comparable data. The IACUC will evaluate whether the species of nonhuman primate proposed for use is the most suitable.

(9) The IACUC will ascertain whether euthanasia of nonhuman primates is necessary as part of the scientific study. If euthanasia is necessary, then the IACUC will determine whether sharing of body tissue to maximize the amount of data obtainable from each animal is possible, thus reducing the overall need among protocols for animals.

(10) Protocols authorizing dogs, cats, and non-human primates, with approved minutes, will be forwarded through the appropriate chain of command, with IACUC documentation, for approval and secondary review in accordance with DOD Directive 3216.1. All Navy, and all Navy funded protocols will be forwarded through HQ, NMRC to the Special Assistant for Veterinary Affairs to the Surgeon General of the Navy. In cases where the appropriate office for secondary review is unclear, the protocol will be directed to the agency providing the majority of the funding.

(11) Approved protocols will be assigned a protocol number. Protocols will be approved for a three-year period.

(12) Annual Protocol Review Reports and updates will be reviewed and approved for the protocol to remain active each year.

#### 11. Protocol:

a. Protocols must use the DOD Standard Protocol Template (Enclosure 1).

b. Protocols by law, by regulation and by the mandate of the IACUC must include specific information. The information required in each protocol is detailed in the protocol template (Enclosure 1). In general, the IACUC is primarily interested in issues of animal care and use, while the PIs are primarily responsible for scientific merit and mission relevance. However, since the approval of animal use is tied to the value

of the information gained, the IACUC may decide on the basis of scientific merit whether the use of the animals is justified. Conversely, Program Directors should consider whether the proposed use of the animals is justified by the scientific merit and mission relevance.

c. Protocols with a pain code of "C" (unrelieved pain or distress) must also prepare and submit the Pain Code Explanation Form shown in Enclosure (3). A protocol can contain more than one USDA code.

(1) Pain codes are "N" (no pain), "D" (deferred or relieved pain through use of appropriate anesthetics and/or analgesics) and "P" (non-relieved pain or distress, usually used where analgesics would interfere with the variable to be measured).

(2) A painful procedure is any procedure, which would be reasonably expected to cause more than slight or momentary pain or distress in a human being to whom that procedure was applied, (i.e., pain in excess of that caused by injections or other minor procedures).

(3) Non-use of drugs to relieve pain must be explicitly justified. Protocols with "P" codes must submit a justification for pain form (Enclosure 3). This form becomes part of the annual USDA report required under the Federal Animal Welfare Act. A USDA Pain justification form must be included with a protocol regardless of the number of animals falling in category "E".

(4) PIs should check with the consulting veterinarian at the time their protocol is prepared with regard to classification of certain procedures and euthanasia methods.

d. Protocols should be scientifically rigorous, yet be written in a clear and logical style. An intelligent reader should be able to understand, in general, why and how the PI is going to use the animals.

e. The protocol should also include a section on safety or hazards if infectious (human or animal), chemical or radioisotope hazards exist. This section should explain in detail how these elements will be managed by the PI.

(1) Protocols should include a section on compliance with DA PAM 385-69, "Biological Defense Safety Program", if Biological Defense programs are involved.

(2) All protocols containing a safety or hazard issue must be signed by the Chair of the Occupational Health Program Chair of the appropriate safety Committee, or office (e.g., Radiation Officer, Bio-safety Committee Chair, or the Safety Office).

f. The protocol should include a section on training and or experience of the PI and associated personnel who will perform the procedures described in the protocol. Training should include experience and knowledge with the procedures and animal species to be used. Specific formal training such as the animal workshops held by Animal Facility Department or similar classes should be cited. IACUC members and/or Animal Facility Department staff may observe a technique performed by an investigator as part of their animal use and training oversight responsibilities.

g. The protocol must also include the verbatim *Assurance Statement* detailed on page 14 of the Protocol Template, followed by the typed name and signature of the PI.

h. Procedures that require surgery must be fully and explicitly described. By law and regulation, very specific rules apply to all animal surgery, especially survival surgeries.

(1) All survival surgery (that from which an animal recovers) must be performed in accordance with applicable laws, regulations, standards, and guidelines

(2) All survival major operative procedures on non-rodents must be performed in a facility dedicated for that purpose (i.e. a surgery room). Major operative surgery is any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions (9 CFR).

(3) Minor operative surgery, non-survival surgery and all surgery on rodents may be performed in a non-dedicated facility (e.g. a portion of the laboratory) but surgery must be performed

away from pedestrian traffic and aseptic conditions must be used.

(4) No animal should be used in more than one major operative procedure from which it is allowed to recover unless the multiple procedures are explicitly justified by the PI in the protocol or the multiple procedures are required as veterinary procedures needed to protect the health of the animal as determined by the attending veterinarian.

(5) A plan for appropriate post-operative care and monitoring must be detailed in the protocol.

(6) According to *The Guide*, "The attending veterinarian must provide guidance or oversight to surgery programs and oversight of post-surgical care," and "The investigator and veterinarian share responsibility for ensuring that post-surgical care is adequate."

i. The use of Freund's adjuvant must be completely described and explicitly justified, since this agent can cause pain and distress and alternative methods are sometimes suitable.

j. The means of euthanasia should be stated. If animals are given CO<sub>2</sub> or lethal drug injection, death must be assured by follow-up heart stab or cervical dislocation after animals are not breathing. The use of decapitation (small animals) or cervical dislocation without anesthesia requires a justification.

k. Investigator sharing of animal tissues is encouraged because it decreases the overall numbers of animals needed. Tissues may be collected at the time of euthanasia.

(1) Use of tissue from dead or dying (during euthanasia) animals should be regarded as a minor modification to an existing protocol.

(2) If the animals are being euthanized by Animal Facility Department personnel, the receiving PI should provide a short description of the material to be received and its proposed use. This information can be approved by the veterinarian and filed by Animal Facility Department.

(3) When the animals are euthanized by the original PI as part of the research procedure, another PI wishing to use tissue from the dead animal should provide the description to the protocol PI directly, who should then include it in his/her annual protocol review update.

(4) Investigators who need to collect animal tissues (including blood) regularly from live animals should prepare protocols for this purpose. Transfer of live animals among approved protocols, (with veterinary review, IACUC approval and proper documentation) is acceptable. The sharing of animals is one way of meeting the Alternatives concept of reduction of animal numbers, and is encouraged by the IACUC, provided such sharing does not violate the restriction against multiple, major survival surgeries on a single animal.

(5) Death-as-Endpoint procedures must be scientifically justified. A plan to provide enhanced care to moribund animals must be included.

## 12 The IACUC Administrator will

a. The IACUC Administrator or IACUC Chair will maintain all records required by law or regulation in accordance with the Animal Welfare Act, USPHS Policy, DOD Directive 3216.1, and SECNAVINST 3900.38B.

b. The IACUC Administrator, upon consultation and approval by the IACUC Chair, shall schedule, coordinate, record and document Committee meetings and inspections; schedule, coordinate, record and document investigator training; maintain protocol files and other Committee records; and assist the IACUC in preparing reports as required by the DOD, USDA, OPRR, AAALAC and the Detachment.

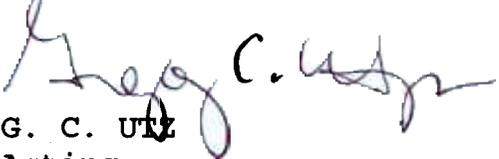
c. The IACUC Administrator or IACUC Chair will maintain records of approved protocols including approved amendments for a minimum of three years after completion of the protocol. The record will include pertinent discussion of the protocol by IACUC members, and the record of approval/disapproval by the IACUC. All records, including procurement data will be kept with the protocol.

d. The IACUC Administrator will maintain records of the minutes of IACUC meetings. These minutes will include the title and PI for each protocol voted upon, and a brief summary of all business discussed at the meetings. The Chair, IACUC, will maintain the file of minutes. The minutes will also include a record of attendance.

e. The IACUC Administrator will maintain records of the IACUC's semiannual Facility Inspection and Program Review inspections and recommendations (including minority views). The inspection reports must contain a description of the nature and extent of the facility's adherence to Federal law and must distinguish between significant versus minor deficiencies. A significant deficiency is one that, with reference to Subchapter A, Title 9, CFR, and in the judgment of the IACUC and the Institutional Official, is or may be a threat to the health or safety of the animals. If program or facility deficiencies are noted, the reports must contain a reasonable and specific plan and schedule for correcting each deficiency. Any failure to adhere to the plan and schedule that results in a significant deficiency remaining uncorrected shall be reported in writing by the IACUC to the Institutional Official.

f. The IACUC Administrator will maintain animal welfare training records on investigative, veterinary, and animal care personnel.

g. On or before December 1 of each calendar year, the IACUC Administrator shall prepare annual reports and submit to the IACUC Chair. The Command shall submit annual reports to the USDA, AAALAC, OLAW and the DoD Animal Care and Use Data Collection Report no later than 30 January of each calendar year. The Institutional Official shall sign all reports.

  
G. C. Uitz  
Acting

**PROTOCOL NUMBER:**  
 Received by IACUC \_\_\_\_\_  
 Approved/Sent for Revision \_\_\_\_\_  
 Received (2nd Time) by IACUC \_\_\_\_\_  
 Date Approved/Disapproved by IACUC \_\_\_\_\_  
**ACTIVE/COMPLETED/TERMINATED**  
 Expiration Date \_\_\_\_\_  
 Previous Number \_\_\_\_\_

**PROTOCOL TITLE:** \_\_\_\_\_

**SHORT TITLE:** \_\_\_\_\_

**PRINCIPAL INVESTIGATOR (Print Rank, First & Last Name)** \_\_\_\_\_

**PI PHONE:** \_\_\_\_\_

**ASSOCIATE INVESTIGATOR:** \_\_\_\_\_

**ASSOCIATE INVESTIGATOR:** \_\_\_\_\_

DEPARTMENT	DIVISION
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APC/JON \_\_\_\_\_ RAD \_\_\_\_\_ STO \_\_\_\_\_ TASK \_\_\_\_\_ 1498 ASSN \_\_\_\_\_

**ANIMAL REQUIREMENTS: (including dams)**

Species	Strain	Age	WT	Sex (M, F, E)	Total Number	Max no. Housed

**IS SPECIAL HOUSING REQUIRED? (YES/NO)** If yes, explain or cite section in protocol:

**ARE ANIMAL REQUIREMENTS RESTRICTED TO A SINGLE VENDOR?**

ESTIMATED ANIMAL CARE COSTS (does not include other costs associated with protocol):

a. Cost of each animal \_\_\_\_\_ X number of animals required \_\_\_\_\_ = \_\_\_\_\_

b. Cost of per diem for animal species \_\_\_\_\_ X number of animals \_\_\_\_\_ X number of days on study \_\_\_\_\_ = \_\_\_\_\_

c. Total of a + b =

**LITERATURE SEARCHES (for unnecessary duplication and 3Rs Alternatives):**

**SEARCH TERMS:**

**DATABASES SEARCHED:**

**DATE OF SEARCH(ES):**

**INDEX KEY WORDS (At least 5 words: e.g. species, strain, condition studied, studied):**

**BIOHAZARD and/or SAFETY ELEMENTS? Y/N (circle) REFERENCE PAGE IN PROTOCOL:**

**BIOSAFETY LEVEL: 1, 2, 3, 4 HAZARDS: a. Chemical b. Radiation c. Infectious Agent  
d. Recombinant Agent e. Select Agents**

**NAME of AGENT(S):**

**USDA PAIN CATEGORY**

**Number of Animals in Category**

**N - Minimal, Transient, or No Pain and Distress**

**D - Pain, Distress Relieved by Appropriate Measures**

**P - Unrelieved Pain or Distress**

**For P, (must be Scientifically Justified) CITE REFERENCE PAGE IN PROTOCOL:**

**ALTERNATIVES CONSIDERATIONS: Does the protocol have any provisions that would qualify it to be identified as one that Refines, Reduces, or Replaces (3R's) the use of animals in relation to other protocols or procedures performed in the past?**

**Y/N (circle) REFERENCE PAGE IN PROTOCOL:**

**PROTOCOL SIGNATURE SHEET:** Requires signature from the Primary Investigator (PI); someone responsible for scientific review (i.e., department or program chief, division or directorate director); the consulting veterinarian; someone responsible for statistical review (i.e., Committee biostatistician); and the Division or Directorate IACUC representative. Coordination signatures from any department providing additional support are required (i.e., Pathology). Coordination signatures from Co-investigators are highly recommended.

**PROTOCOL TITLE:**

**PRINCIPAL INVESTIGATOR:** \_\_\_\_\_

**ASSOCIATE INVESTIGATOR(S):** \_\_\_\_\_

**SCIENTIFIC REVIEW:** Signature verifies that this proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

\_\_\_\_\_  
(Department or Program Chief's/Division or Directorate Director's signature)

**COORDINATION:** Name, signature, and date for the appropriate person or office are required. (No response is required to the title paragraph of this section)

**A. Attending/Consulting Veterinarian:** The attending or consulting veterinarian has reviewed the protocol and was consulted in the planning of procedures that require veterinary input, i.e., an unalleviated pain procedure. In addition, the veterinarian or veterinary medicine department has assisted with coordination for veterinary support to the protocol.

\_\_\_\_\_  
(Attending/Consulting Veterinarian)

**B. Statistician:** A person knowledgeable in statistics has reviewed the experimental design (i.e., the Committee Biostatistician).

\_\_\_\_\_  
(Statistician)

**C. IACUC Representative:**

\_\_\_\_\_  
(IACUC Member Signature)

**D. Occupational Health Review:**

\_\_\_\_\_  
(WRAIR/NMRC Safety Representative)

**E. Other:** The individual(s) whose departments you'll be coordinating with for purposes pertinent to your protocol. *Biosafety Officer signature is required for protocols in which select agents, recombinant agents and/or BSL-3 agents are used.*

\_\_\_\_\_

**(Start New Page Here)**

**PROTOCOL TITLE:**

**PRINCIPAL INVESTIGATOR:**

**ASSOCIATE INVESTIGATOR (S):**

**I. NON-TECHNICAL SYNOPSIS:** Provide a brief, narrative description of the proposal or idea that is easily understood by non-scientists.

**II. BACKGROUND:**

**A. Background:** Include a brief statement of the requirement or need for the information being sought. Lengthy explanations are not required. Typically, the "literature or the experience that led to the proposal will be briefly reviewed" (AR 70-18), and a description of the general approach will be provided. Unnecessary duplication of effort will be strictly avoided.

**B. Literature Search:** A literature search must be performed to prevent unnecessary duplication of previous experiments. A search of the Biomedical Research Database (BRD) is required (website <http://dticam.dtic.mil/dodbr/index.html>). In addition, a search of EITHER the Federal Research in Progress (FEDRIP) or the Computer Retrieval of Information of Scientific Projects (CRISP) is required. Requirements for additional searches are at the discretion of the IACUC. Further searches of the scientific literature (MEDLINE, GRATEFUL MED, MEDLARS, AWIC, etc.) are highly recommended. Please see the reference librarian in the WRAIR Library for assistance.

**Literature Source(s) Searched:**

**2. Date and Number of Search:**

**3. Key Words of Search:**

**4. Results of Search:** Provide a narrative description of the results of the literature search.

**III. OBJECTIVE/HYPOTHESIS:** In non-technical terms, state the objective of this protocol, or the hypothesis to be accepted or rejected.

**IV. MILITARY RELEVANCE:** Provide a brief and succinct military justification for the research with regard to military needs and mission requirements. If applicable, state the Science and Technology Objective (STO) that this work supports.

**V. MATERIALS AND METHODS:**

**A. Experimental Design and General Procedures:** Provide a complete description of the

proposed use of animals. Succinctly outline the formal scientific plan and direction for experimentation. If several experiments or sequential studies are to be included in the protocol, describe the experimental design for each separate experiment in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. However, **a clearly understandable description of the numbers of animals and their distribution into experimental groups is essential.** The number requested should be the minimum numbers necessary to complete the study, but must be sufficient to yield meaningful results. Importantly, if too few animals are used statistical significance will not be achieved, and results of the study may be compromised. Be certain to include animals necessary for controls or technique development, etc. If the design is complex, include a summary table or flow chart showing the distribution of animals by experimental group. **The total number of animals required for the study is listed in section V.C.4. It is critical that reviewers of this protocol are able to follow the reasoning and calculations for the number of animals required, and can verify that the experimental design clearly supports the number of animals requested.**

1. **Experiment 1:**

2. **Experiment 2:**

**B. Data Analysis:** List the statistical test(s) planned or describe the strategy intended to evaluate the data. Consult with the committee biostatistician if necessary.

**C. Laboratory Animals Required and Justification:** Personnel in the animal care unit will assist P.I.s in the preparation of the protocol sections dealing with animal care issues. No response is needed under the title heading of this paragraph.

1. **Non-animal Alternatives Considered:** State whether alternatives to animal use were considered. No study using animals will be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means, for example, computer modeling, cell cultures, etc.

2. **Animal Model and Species Justification:** It is important that the selection of this animal model is adequately justified. Investigators will use the least sentient species that will permit the attainment of research objectives. State why this particular animal was chosen; if other animal models were considered that are lower on the phylogenetic scale (for example, mice instead of rabbits); and whether there a unique quality or usefulness about this species that warrants its selection for use.

3. **Laboratory Animals:** No response necessary to the title paragraph of this section.

a. **Genus & Species:**

b. **Strain/Stock:** If inbred or specialized animals are required, use proper terminology. (Division of Veterinary Medicine (DVM) personnel will provide complete nomenclature for inbred strains and outbred stocks of rodents.)

**c. Source/Vendor:** Provide a preferred source for the animals. Procurement of animals from non-USDA licensed sources requires an exception to policy (see DVM personnel for any assistance required).

**d. Age:**

**e. Weight:**

**f. Sex:**

**g. Special Considerations:** List specialized requirements for the research animals here, for example, simian immunodeficiency virus or herpes antibody free, Pasteurella free, etc.

**h. Other:**

**4. Number of Animals Required (By Species) and Rationale for the Appropriateness of the Number(s):** The number of animals requested here will match exactly those described in section V.A.. A power-based assessment of the sample size is the preferable method of determining the minimum number that is likely to yield significant results with given alpha and beta errors, and estimated effect size and expected variability. Be certain to include animals necessary for controls or technique development, etc. It is critical that reviewers of this protocol are able to follow the reasoning and calculations for the number of animals required, and can verify that the experimental design clearly supports the number of animals requested. Three examples are listed below. If additional animals are needed due to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval for additional animals.

**a. Example of justification for 320 mice:** Twenty animals per group will be used to evaluate 15 compounds. One group of 20 animals will be used as controls. A power-based assessment of the sample size indicates that 20 animals per group is the minimum number that is likely to yield significant results with an acceptable alpha error probability of 0.05 (two-tailed), a calculated beta error of 0.20 or less, an effect size of 25 percent, and expected variability of less than 5 percent.

**b. Example of justification for 175 chinchillas:** Approximately 175 chinchillas will be required for the planned experiments (10 animals per group). This number includes a cull rate of 25 percent to account for animals lost through surgical failure, electrode rejection, pre-existing hearing loss, and other loss of subjects, consistent with experience in this laboratory with similar experiments.

**c. Example of justification for number of animals per group:** The number of animals per group was determined through power analysis (Kirk, 1984), with the aim of achieving adequate sensitivity to detect meaningful treatment differences with as few animals as possible. Kirk (1984) relates the required sample size to the population "effect" size (?-??),

expected error variance( $\sigma^2$ ), desired power ( $1-\beta$ ), and the criterion for significance( $\alpha$ ) in the following manner:  $n = \frac{(z_{\alpha} + z_{\beta})^2 \sigma^2}{\delta^2}$ , where  $z_{\alpha}$  is the z score that cuts off the  $\alpha$  region of the sampling distribution of  $\bar{x}$  and  $z_{\beta}$  is the z score that cuts off the  $\beta$  region of the sampling distribution of  $\bar{x}$ . The following values were used in the sample size determination: A difference of 6dB or more would be considered to be a meaningful difference. Based on results obtained in past studies and the resolution of the measurement, error variance was estimated to be around 7 dB. Using a criterion level of 0.05 and a “moderate” power of 0.80, the minimum sample size was calculated to be 10 animals per group. That is, by using 10 animals per group, one can be assured of being able to correctly reject the null hypothesis (that is, detect a meaningful difference of at least 6 dB) between groups 80 percent of the time.

**5. Refinement, Reduction, Replacement:** DOD is often required to provide specific examples of its alternatives initiatives. State whether this protocol has any provisions that would qualify it to be identified as one that refines, reduces or replaces (3Rs) the use of animals. For example, indicate whether the study uses statistical tests that require fewer animals, such as a modified LD50 test like Thompson and Weil operation, or whether it uses cell cultures, computer modeling or any other technique that will influence the numbers of animals required. State whether animals are used which are lower on the phylogenetic scale. Provide a short description of the features that qualify the study as one that employs one of the “3 Rs,” or give a negative reply. No response is needed under the title paragraph of this section

**a. Refinement:** Examples of refinement include, but are not limited to the use of analgesia, the use of remote telemetry, or the use of adjusted early endpoints. In addition to listing the refinements that will be used, **list the refinement alternatives that were considered but not adopted, and explain why they were not adopted.**

**b. Reduction:** Examples of reductions are the use of shared or historical control groups, preliminary screening in non-animal systems, and innovative statistical packages.

**c. Replacement:** Examples of replacements are non-animal systems that eliminate the use of animals.

**D. Technical Methods:** These will be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure and obtain a clear understanding of what is to be done and how the animal will be handled, and make a reasonable determination as to whether this proposed use of laboratory animals is in compliance with DOD regulations, guidelines, and Federal law. No response is needed under the title paragraph of this section.

**1. Pain:** The law defines a painful procedure as one that would “reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures.” **If a procedure involves pain or distress, the P.I. must consult with the attending veterinarian.** No response is required under the title paragraph of this subsection.

**a. Pain Category (APHIS Form 7023):** This information is reported by the organization to the USDA on APHIS Form 7023. The P.I. or primary user will estimate the number of animals that will be counted in each pain category. There are many situations where there are animals in more than one category, that is, control animals. If more than one species is requested in the proposal, reflect those animals in a duplicate table in this paragraph. The total numbers reflected in these three categories will add up to the number of animals requested for the entire protocol in paragraph V.B.4.

(1) **No Pain:** examples are studies involving no pain or distress beyond that expected on a momentary nature such as would occur with an injection, a deep palpation, grooming activities, etc.

(#) (Column C)

(2) **Alleviated Pain:** These are procedures where anesthesia or analgesia will be administered to avoid or alleviate pain or distress. General anesthesia given for surgical preparations, or the use of analgesia or anti-inflammatories are examples of this category.

(#) (Column D)

(3) **Unalleviated Pain or Distress:** These are procedures where alleviation of pain or distress are contraindicated for a justifiable reason such as “would confound the experimental results if drugs relieving pain were administered (explain specifics).” Detailed justification for putting animals into this category is required below in paragraph V.C.1.d.

\_\_\_\_\_ (#) (Column E)

**b. Pain Alleviation:** The attending veterinarian will provide assistance in completing this section of the proposal. No response is necessary under the title paragraph of this subsection.

(1) **Anesthesia/Analgesia/Tranquilization:** Describe the methods or strategies planned to alleviate pain or distress if the study will cause more than temporary pain or discomfort. If pain alleviation is planned, specify **who** will be administering the analgesics, anesthetics, or tranquilizers during the study. Provide agent, dosage, route and site, indication, needle size, etc.

(2) **Paralytics:** The use of paralytic agents without anesthesia is prohibited unless scientifically justified by the P.I. and approved by the IACUC.

**c. Alternatives to Painful Procedures:**

(1) **Source(s) Searched:** Examples are AGRICOLA, MEDLINE, AWIC, etc.

(2) **Date of Search:**

(3) **Key Words of Search:** Examples are pain, surgery, etc.

(4) **Results of Search:** Respond N/A if the animals will experience “no pain or distress.” Otherwise, provide a narrative description of the results of the alternatives literature search. Research facilities will be held responsible, if it is subsequently determined that an alternative to a painful procedure was available to accomplish the objectives of the proposed experiment. The Animal Welfare Act specifically states that the **“P.I. must provide a narrative description of the methods and sources, e.g., the Animal Welfare Information Center, MEDLINE, Life Sciences Abstracts, AGRICOLA, AND BIOSIS that he/she used to determine that alternatives to the painful procedure were not available.”** The alternatives literature search and painful procedure justification must be performed even when animals are placed in the alleviated pain category (column D).

**d. Painful Procedure Justification:** Respond N/A if the animals will experience “no pain or distress.” Otherwise, procedures causing more than transient or slight pain must be justified on a scientific basis in writing by the P.I. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized. This paragraph must be completed if there are any animals listed in either the alleviated (column D) or the unalleviated pain or distress (column E) category in paragraphs V.C.1.a.(2) and (3). **The P.I. must consult with the attending veterinarian or his or her designee in the planning of both alleviated and unalleviated painful procedures, and state so here.** The following paragraph may also be included in this category: “Although, by necessity, there are animals included in the unalleviated pain and distress category on this protocol, there will be a conscious effort by the P.I. and animal care staff to provide as much additional consideration for the comfort and well-being of the animals as is consistent with the scientific integrity of the study.”

**2. Prolonged Restraint:** Describe and justify in detail any prolonged restraint (greater than twelve hours) intended for use during the study, for example, primate chairs, restraint boards, metabolism cages, etc. Also describe habituation procedures for the prolonged restraint. This section is not intended for short-term actions such as rabbit restraint for bleeding, etc. If there is prolonged restraint involved, who will restrain the animals, and for how long. Refer to WRAIR Policy Letter 00-07, “IACUC Guidelines on Prolonged Restraint” for further details.

**3. Surgery:** Major operative procedures on non-rodent species, for example, rabbits, monkeys, etc., will be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions. Non-major operative procedures and all rodent surgery do not require a dedicated facility, but **must be performed** using aseptic technique, that is, surgical gloves, mask, and sterile instruments. A major operative procedure is one that penetrates and exposes a body cavity, or causes permanent impairment of physical or physiological function. The animal care unit personnel should assist in defining the requirements of this portion of the law if necessary. Refer to WRAIR Policy Letter 00-08, “IACUC Guidelines for Surgery and Recovery for Rodents” for further information. No response required under the title paragraph of this section.

**a. Procedure:** Describe in detail any surgical procedures planned.

**b. Pre-and Postoperative Provisions:** Detail the provisions for both pre-and postoperative care, including provisions for post-surgical observations. Also include the provider of that care, and the location for the postoperative care. Describe the plans for pain alleviation after surgery and the monitoring plan until animals have fully recovered from anesthesia. This is required for all surgeries performed at WRAIR/NMRC, including rodent surgeries. If analgesia cannot be used post-operatively, it must be scientifically justified for any surgery.

**c. Location:** Give the location/room number for the proposed surgical procedure.

**d. Surgeon/Qualifications:** Indicate who will perform the surgery, and his/her qualifications, training, or experience in the proposed procedure. The attending veterinarian may provide training and verification of experience in surgical, technical, and handling procedures, if necessary.

**e. Multiple Survival Surgery Procedures:** Multiple major operative procedures on the same animal must be scientifically justified for scientific reasons by the P.I. in writing. Refer to WRAIR Policy Letter 00-16, "IACUC Guidelines for Multiple, Major Survival Surgeries" for more information.

**(1) Procedures:**

**(2) Scientific Justification:**

**4. Animal Manipulations:** Describe any injections, sampling procedures, or other manipulations of the animals necessary for the execution of the study. List needle sizes, routes of injection or withdrawal, and anatomical location for example, 21 gauge needle, subcutaneous, intramuscular, femoral vein, jugular vein etc., or the proposed method, so that a reasonable evaluation of the appropriateness of the procedure can be made. A reference or SOP may be furnished to the IACUC to document a particular procedure in lieu of a detailed description. No response is needed under the title paragraph of this section.

**a. Injections:** There is no need to duplicate specific information already provided in paragraph V.C.1.b.

**b. Biosamples:** Examples are cerebral taps, blood sampling, etc. List amounts taken and method for sampling. Procedures performed or biosamples obtained during a necropsy should not be described here. Refer to the blood volume chart in the Investigator's Handbook for more specific details on appropriate blood volumes to be drawn, based on species and size.

**c. Animal Identification:** Examples are microchips, tattoos, ear tags, cage cards, etc.

**d. Behavioral Studies:** Fully describe any intent to use aversive stimuli, food or water deprivation, etc., that would impact upon the animals in the study, and include appropriate justification for this requirement.

**e. Other procedures:** These may include electrocardiograms, radiology, aerosol exposure, etc. (include appropriate information, e.g. SOP numbers, where applicable).

**5. Adjuvants:** List any adjuvants and plan for their use. Provide dosages and route, and specify that the injection sites will be monitored in accordance with the adjuvant policy, and that the attending veterinarian will be consulted if there is any adverse reaction to the adjuvant. Please refer to WRAIR Policy Letter 00-03, "IACUC Guidelines for the Use of Complete Freund's Adjuvant and Ascites Priming Agents/Production in Laboratory Animals" for further information.

**6. Study Endpoint:** State the projected endpoint or termination of the study for the animals. Indicate whether death, euthanasia, or recovery is expected; and the specific plan for determining when the animal experimentation phase will be stopped. The P.I. will ensure that unnecessary pain or distress is prevented by carefully considering "When is the experimental question answered?" so that the animals can be removed from the study as soon as feasible. Explain the plan for the disposition of surviving animals. **Specifically address and justify any proposed use of death as an endpoint.**

**7. Euthanasia:** Explain the plan for euthanasia of the animals at the completion of the study and indicate who will perform the procedure. The Animal Welfare Act (AWA) defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current American Veterinary Medical Association (AVMA) guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. Exceptions must be scientifically justified by the P.I. in writing. The attending veterinarian will assist in selecting the best method for euthanasia if requested. The current AVMA euthanasia guidelines are available on the Animal Care and Use web page on the WRAIR website [wrair-www.army.mil](http://wrair-www.army.mil).

**E. Veterinary Care:** Attending veterinary care of lab animals receives particular emphasis in the AWA. The attending veterinarian of the facility will assist P.I.s with preparing this section if requested. No response is necessary to the title paragraph of this subsection.

**1. Husbandry Considerations:** The law specifically states that animal housing and living conditions must be appropriate to their species, and contribute to their health and comfort. If the cages in the containment facilities cannot be removed and sanitized according to the *Guide for Care and Use of Laboratory Animals* or the DVM SOP, this requires an exception that must be approved by the IACUC: therefore, include this information in the protocol. Any deviation from the approved space requirements of feeding and watering for animals as specified in the *Guide* must also be approved by the IACUC. Indicate in this paragraph that "the animals will be

observed daily for general health, humane treatment, and husbandry considerations.”

a. **Study Room:** This applies if the stay exceeds 12 hours.

b. **Special Husbandry Provisions:** Examples are micro-isolators, metabolic cages, etc.

**2. Attending Veterinary Care:** State if the animals will be observed daily or more frequently, and by whom. Indicate what will happen if the animal becomes ill or debilitated during the study and requires supportive therapy. State if the animal will be euthanized if it becomes critically ill or comatose, and by whom (study end point adjustment). Justification for not providing supportive care for clinically ill animals is necessary.

**3. Enrichment Strategy:** Provide written justification for restricting enrichment programs or activity programs of dogs, cats, or non-human primates. Single housing of non-human primates and dogs without sensory contact with conspecifics must also be justified and approved by the IACUC. All animals participate in an enrichment program at WRAIR/NMRC. No response is necessary to the title paragraph of this subsection.

a. **Dogs:** If there is any reason to restrict activity programs for dogs on this protocol that might be implemented by the animal care unit to comply with Federal welfare regulations, provide justification.

b. **Non-human Primates:** If there is any reason to prohibit environmental enrichment or enhancement strategies that might be implemented by the animal care unit to comply with Federal welfare regulations, provide justification.

**VI. INVESTIGATOR AND TECHNICIAN QUALIFICATIONS/TRAINING:** List those animal procedures or manipulations described in the protocol that will be performed by each investigator or technician, and their training or qualifications to perform these procedures. Personnel conducting the “hands-on” animal procedures described in the protocol must be identified and appropriately trained and qualified to perform these procedures. This is questioning the P.I.’s professional qualifications to conduct the research, but rather a requirement that personnel actually performing the research animal manipulations are technically competent, and thus are not inflicting unnecessary pain, distress, or injury to an experimental animal due to inexperience or improper technique. Contact the attending veterinarian for assistance with this requirement. Refer to WRAIR Policy Letter 00-09, “IACUC Requirements for Personnel Qualifications and Training” for more information. WRAIR/NMRC offers general species-specific courses and an Animal Care and Use Program Orientation class for those who have not met this training requirement. Contact the IACUC Activities Office for more information on class availability.

<u>Name</u>	<u>Procedure</u>	<u>Training</u>	<u>Qualifications</u>
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**A. Investigator(s):**

**B. Technician(s):**

**VII. BIOHAZARD/SAFETY:** Provide a list of any potential biohazards associated with this proposal, for example, viral agents, toxins, radioisotopes, oncogenic viruses, chemical carcinogens, etc. Explain any safety precautions or programs designed to protect personnel from biohazards, and any surveillance procedures in place to monitor potential exposures. Some BSL-2 and recombinant DNA protocols and all BSL-3 protocols must be reviewed and approved by the Institute Biosafety Committee (IBC). Contact Dr. David Lanar, Chair, to inquire whether a protocol will require this approval. If IBC approval is required, then the appropriate documentation must be included along with the protocol.

**(Start New Page Here)**

**VIII. ASSURANCES: As Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:** The law specifically requires several written assurances from the PI. It states that “research facilities will be held responsible if it is subsequently determined that an experiment is unnecessarily duplicative, and that a good faith review of available sources would have indicated as much.” No response is required under the title paragraph of this section.

**A. 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 deviation is specifically approved by the IACUC.

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

**C. Statistical Assurance:** I assure that I have consulted with a qualified statistician to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals needed for scientific validity are used.

**D. Biohazard\Safety:** I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

**E. Training:** I verify that the personnel performing the animal procedures/manipulations 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 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 conduct this study in the spirit of the fourth “R” which the DoD has embraced, namely, “Responsibility” for implementing animal use alternatives where feasible, and conducting humane and lawful research.

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(P.I. Signature)

**G. Painful Procedures:** (A signature for this assurance is required by the P.I. only if the research being conducted will cause more than slight or momentary pain or distress. See paragraphs V.C.1.a.(2) and (3), column D or E by USDA classification. If painful procedures are not being conducted in this protocol, please type N/A in the signature block.) I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that **WILL or WILL NOT (circle one)** be relieved with the use of

anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

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(P.I. signature)

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

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(P.I. signature)

**IX. ENCLOSURES:** This space is available for the attachment of any documents such as literature searches, SOPs, references, or other information pertinent to the protocol. Examples are as follows:

**A. Literature Searches:** Examples are DTIC, MEDLINE, AGRICOLA, etc.

**B. Pathology Addendum:** Optional information can be added here.

**C. Pain Assessment Guidelines:**

**D. Adjuvant Policy:**

# Animal Use Protocol, Annual Review Form – FY04

## Naval Medical Research Center Detachment

**PROTOCOL TITLE:**

**PROTOCOL #:**

**INITIAL APPROVAL DATE:**

**PRINCIPAL INVESTIGATOR:**

**PROGRAM:**

**ROOM NUMBER:**

**PHONE NUMBER: EXTENSION**

**FAX NUMBER:**

**E-MAIL ADDRESS:**

1. **FUNDING** Specify funding source, total funding amount, and funding expended for this fiscal year (total best estimate for animals, supplies, equipment, per diem, etc.).
2. **WORK UNIT INFORMATION:** Major Research Category (circle one) supported by this protocol:
3. **OBJECTIVE** – Briefly state objective/hypothesis for your protocol that is easily understood by non-scientists (1-4 sentences).
4. **NON-TECHNICAL SYNOPSIS** - Provide a brief summary that is easily understood by non-scientists that summarizes the protocol (1-4 sentences) in non-technical terminology. This synopsis will be indexed in the BRD database.
5. **PROTOCOL STATUS** – (Check applicable category.)  
Request Protocol Continuance
  - A. Active – project ongoing.
  - B. Currently inactive – project was initiated but is presently inactive.
  - C. Inactive – project never initiated but anticipated start date is \_\_\_\_\_

**Request Protocol Termination**

- D. Inactive – project never initiated.
- E. Currently inactive – project initiated but has not/will not be completed.
- F. Animal use completed, data analysis or human work still pending.
- G. All work has been completed. A final report will be submitted to the Chair, Institutional Animal Care and Use Program.

**6. RECORD OF ANIMAL USE BY PAIN CATEGORY:**

a. Are current animal use procedures essentially the same as described in the protocol when it was last approved, or within approved amendments to the protocol?

\_\_\_\_\_ Yes \_\_\_\_\_ No (Please explain any changes)

b. Indicate the species and number used per USDA Pain Category thus far in FY 04 in the table below. Please estimate the number of animals to be used in each Pain Category through 30 September 2004:

USDA CATEGORY	Species and Number Used this FY to Date (01OCT 03-30 SEP 04)	Estimated Number Used for Remaining FY 04
N - Minimal, Transient, or No Pain and Distress		
D - Pain, Distress Relieved by Appropriate Measures		
P- Unrelieved Pain or Distress		
<i>Total this FY</i>		
<i>Total used under this protocol</i>		
<i>Total approved for use under this protocol</i>		

c. For categories D and P in the above table, have alternatives to painful procedures been considered in preparation of this protocol, and implemented where possible, consistent with the scientific integrity of the study.

\_\_\_\_\_ Yes \_\_\_\_\_ No

d. For category P in the above table, unrelieved pain or distress, provide an explanation of the procedures producing pain or distress and the reasons analgesics, anesthetics, or tranquilizing drugs were not used.

e. In your judgment, has the current level of pain and discomfort changed from when the study was first approved?

\_\_\_\_\_ Yes \_\_\_\_\_ No (Please explain any changes in pain level)

f. Were there any unanticipated adverse events, morbidity or mortality. If so, provide an explanation.

\_\_\_\_\_ Yes \_\_\_\_\_ No

g. Does this protocol incorporate any exceptions to animal welfare standards and regulations? If so provide a brief explanation of the exception, its justification, and the number of animals affected.

\_\_\_\_\_ Yes \_\_\_\_\_ No

**7. PROGRESS AND 3Rs/ALTERNATIVES WORK:**

a. Does this protocol currently demonstrate any of the 3Rs/Alternatives applications as listed below?

\_\_\_\_\_ Yes \_\_\_\_\_ No

b. If yes, please indicate Alternatives involved. ("X" all that apply):

**Refinement**

- \_\_\_ protocol uses a pilot study to refine techniques and/or define the animal model
- \_\_\_ protocol collects several types of data simultaneously
- \_\_\_ protocol uses environmental enrichment strategies
- \_\_\_ protocol participates in an environmental enrichment program that included food treats, toys, group housing, and/or positive interaction with humans
- \_\_\_ protocol uses non-lethal endpoints
- \_\_\_ protocol incorporates the use of anesthesia to reduce or alleviate the associated pain and discomfort to the animal
- \_\_\_ protocol incorporates the use of analgesics to reduce or alleviate the associated pain and discomfort to the animal
- \_\_\_ other \_\_\_\_\_

**Reduction**

protocol eliminates animal use  
protocol uses non-animal training aids  
protocol involves using animals that serve as their own control  
protocol involves sharing animal tissues and/or control groups with other investigators that had different protocols  
protocol performs statistical analyses to assure the least number of animals necessary are used to achieve significance  
other \_\_\_\_\_

**Replacement**

\_\_\_\_\_ uses procedures that fully replace the need for animal use  
\_\_\_\_\_ computer modeling  
\_\_\_ substitution of a lower species than previously used

Original Species: \_\_\_\_\_ Alternative Species or Model: \_\_\_\_\_

substitution of insentient material and *in vitro* biological systems for animals  
tissue culture  
other \_\_\_\_\_

8. Provide specific examples of **definitive program benefits** directed toward meeting defined military and research requirements or dual use applications that were dependent on animal use from your protocol. (Bullet comments)

9. Provide specific examples of **definitive program accomplishments** directed toward meeting defined military and research requirements or dual use applications that were dependent on animal use from your protocol. (Bullet comments)

10. **PUBLICATIONS, PRESENTATIONS & AWARDS** – List the recent (FY04) information for the following: journals, (include number, volume, name and pages of publications) (P), titles and status of manuscripts (M), organization, location and date of presentations (Pr) and similar information about abstracts (A) resulting from this study. List also any significant awards and/or special recognition received as a result of work on this study. Please provide the complete and recent information for all or any of the above (i.e. name, number, date, pgs, volume and title).

11. **SURGICAL PROCEDURES** – If the protocol involves any surgical procedure, provide the following information: surgical procedures performed, anesthesia used, analgesia given, post-operative follow-up and care.

- a. Surgical Procedures Performed
  - 1)
  - 2)

- 3)
- 4)

b. Anesthesia Used

- 1)
- 2)
- 3)
- 4)

c. Analgesia Used

- 1)
- 2)
- 3)
- 4)

d. Post-op Care

- 1)
- 2)
- 3)
- 4)

12. Does this protocol require special safety considerations for animal care and research personnel? (e.g. infectious agents, radioactive agents, interaction with macaques)

Yes                      No

If yes, please describe the safety considerations and training and/or safety briefings provided to personnel).

13. **ANIMAL RECORDS** – Take the time to update your animals' health (Veterinary Treatment Record, SF 600), located in the Animal Facility Department staff office. Animals such as dogs, pigs and nonhuman primates are usually used in more than one research project and updated health records, reflecting research-induced conditions, help determine future animal-protocol assignments. Additionally, research procedures and surgical manipulations should be recorded for all rodent work. Information should be readily available, and kept either in the PI's notebook or the animal room.

14. **OCCUPATIONAL HEALTH AND TRAINING** – List the last occupational health review for each Principal Investigator, Associate Investigators and technicians (to include contractors) who worked on this protocol. This is an annual requirement. Please be sure to update your Occupational Health Records with the NMRCO Occupational Health Manager, CDR Gregory Utz at 562-3848, extension 113. Also list animal care and use training courses that personnel active on this protocol have taken during this fiscal year only.



Protocol Number:

**USDA Form 18-23**

**PROJECT TITLE:**

**PROTOCOL NUMBER:**

Number and species of animals listed in Column D or P. Use separate form for each category and/or species.

**SPECIES:**

**NUMBER:**

**BRIEF DESCRIPTION OF PROJECT:**

**EXPLANATION OF RELIEVED PAIN OR DISTRESS (Column D):**

**EXPLANATION OF UNRELIEVED PAIN OR DISTRESS (Column P):**

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**SIGNATURE/DATE OF PRINCIPLE INVESTIGATOR**

**Enclosure (3)**

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