

APPENDIX A

DoD DIRECTIVE ON ANIMAL USE



Department of Defense DIRECTIVE

April 17, 1995
NUMBER 3216.1

DDR&E

SUBJECT: Use of Laboratory Animals in DoD Programs

- References:
- (a) DoD Directive 3216.1, "Use of Animals in DoD Programs," February 1, 1982 (hereby canceled)
 - (b) Title 9, Code of Federal Regulations, "Animals and Animal Products," Chapter 1, Subchapter A, "Animal Welfare," Parts 1, 2, and 3
 - (c) Public Law 101-511, Department of Defense Appropriations Act for Fiscal Year 1991, Section 8019, Title 10 United States Code, Section 2241
 - (d) Sections 2131 through 2156 of Title 7, United States Code "The Laboratory Animal Welfare Act of 1966," as amended
 - (e) through (f), see enclosure 1.

A. REISSUANCE AND PURPOSE

1. Reissues reference (a) to update policy governing activities using animals within the Department of Defense.

2. Designates the Secretary of the Army as the DoD Executive Agent to develop and issue Service regulations to implement this Directive.

B. APPLICABILITY

This Directive applies to the Office of the Secretary of Defense, the Military Departments, the Uniformed Services University of the Health Sciences, and the Defense Agencies (hereafter referred to collectively as "DoD Components") that perform or sponsor activities using animals.

C. DEFINITIONS

Terms used in this Directive are defined in enclosure 2.

D. DoD POLICY

1. Federal statutes, regulations, and publications that provide national standards and guidance for the acquisition, transportation, housing, control, maintenance, handling, protection, treatment, care, use, and disposal of animals shall be applicable to all activities using animals. A summary of the applicable documents cited as references is in enclosure 3.

2. Animals shall be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with

reference (b) unless specifically exempted from the licensing requirements stated in reference (b).

3. DoD organizations or facilities maintaining animals for use in research, testing or training shall apply for accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

4. Alternative methods to animal species shall be considered, whenever possible, if such alternatives produce scientifically valid or equivalent results to attain the research testing and training objectives.

5. The purchase or use of dogs, cats, or nonhuman primates in research conducted for developing biological, chemical or nuclear weapons is prohibited.

6. The purchase or use of dogs, cats, or nonhuman primates for inflicting wounds from any type of weapon(s) to conduct training in surgical or other medical treatment procedures is prohibited. (reference (c)).

7. DoD organizations or facilities wishing to hold training programs using animals, such as advanced trauma life support (ATLS) training programs, shall have the training protocol reviewed and approved by a duly constituted Institutional Animal Care and Use Committee (IACUC) in accordance with references (d) and (e) and paragraph D.8. of this Directive to ensure the humane use of animals. DoD organizations or facilities conducting ATLS training that require housing of animals for short periods of time shall ensure adequate care and shall have the animal housing facilities inspected and approved by a veterinarian prior to receipt of the animals.

8. All proposals or protocols for animal experiments or demonstrations in RDT&E, clinical investigation, instructional, or training programs conducted or sponsored by a DoD organization or facility shall be reviewed and approved by a duly constituted IACUC composed of a minimum of five members. There shall be at least one non-scientific member on each IACUC. In addition, there also shall be a member who represents the general community interest and is non-affiliated with the facility sponsoring IACUC. The non-affiliated and the non-scientific membership can be filled by the same person. To ensure community representation at each meeting and inspection, an alternate to the non-affiliated member shall be designated for IACUCs having a single non-affiliated membership. Since the DoD IACUCs perform a Government function in an approval process and do not serve merely as an advisory body, the non-affiliated and the non-scientific member(s) to DoD IACUCs shall either be a Federal

employee, with demonstrated commitment to the community or a consultant consistent with the requirements established by reference (f).

9. A headquarters-level administrative review shall be conducted for proposals involving the use of non-human primates conducted or sponsored by subordinate activities of the DoD Component for conformance with all applicable Federal regulations and policies. A DoD component may delegate this responsibility to another DoD component for purposes of efficiency and consolidation of functional offices.

10. The DoD Components shall coordinate and cooperate in the transfer of Government-owned nonhuman primates between facilities to maximize conservation and proper utilization.

11. Proposals intending to use chimpanzees must be further reviewed and approved by the Interagency Animal Model Committee, which coordinates national priorities for research utilization of this species.

12. The DoD components that sponsor animal based research, testing, and training under a DoD grant or contract shall ensure that:

a. all extramural research proposals using live animals shall be administratively reviewed by a DoD veterinarian trained or experienced in laboratory animal science and medicine before grant or contract award.

b. the most recent USDA inspection reports are provided or obtained for the facility under consideration for a research contract or grant using animals, and that during the term of the award, the most recent USDA inspection reports be reviewed on an annual basis.

c. a DoD veterinarian trained or experienced in laboratory animal science and medicine shall conduct an initial site visit to evaluate animal care and use programs at contracted facilities conducting DoD-sponsored research using non-human primates, marine mammals, dogs, cats, or proposals deemed to warrant review. The initial site visit shall occur within 6 months of when the facility has taken delivery of the animals under DoD contract or grant award. Any facility receiving a DoD-funded grant or contract for animal based research shall notify the DoD component sponsor and shall have a site inspection within 30 days of notification of loss of AAALAC accreditation for cause, or notification that the facility is under USDA investigation. Site inspections for cause shall evaluate and

ensure the adequacy of animal care and use in DoD-sponsored programs, and provide recommendations to the sponsoring DoD component about continued funding support of the research.

13. In the case of differences between the standards of care and use of animals as cited in enclosure 3, the most stringent standard shall apply.

14. Activities covered by this Directive that are performed or sponsored in foreign countries shall be conducted in accordance with applicable U.S. statutory requirements, and regulations and standards of the host country. If differences exist between U.S. and host country regulations or standards, unless prohibited by the host country, the more stringent standard shall apply.

15. While not specifically addressed in this Directive, ceremonial, recreational, and working animals, such as military working dogs, shall be treated in a humane manner.

16. Personnel with complaints of violation of this directive shall report such violations to either of the following members of the organization or facility: IACUC chairperson, attending veterinarian, the facility Commander, or Inspector General. The IACUC shall review and, if warranted, investigate all reports of complaints of animal use or noncompliance with 7 U.S.C. 2131-2 of reference (d), applicable Directives, and regulations.

E. RESPONSIBILITIES

1. The Director, Defense Research and Engineering (under the Under Secretary of Defense for Acquisition and Technology) or designee shall:

a. Issue policy and procedural guidance concerning animal use consistent with all applicable Federal regulations and policies.

b. Designate a DoD representative to the Interagency Research Animal Committee who is a veterinarian of appropriate rank or grade and experience, and preferably also a diplomate of the American College of Laboratory Animal Medicine.

c. Establish the Joint Technical Working Group (JTWG) to act as the central advisory committee to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee on all matters on the care and use of animals for research, testing, clinical investigation, or training within the Department of Defense. The co-chairpersons of the ASBREM Committee shall designate the chairperson of JTWG.

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2. The Heads of the DoD Components shall:

a. Establish appropriate mechanisms to monitor compliance with this Directive and applicable Federal statutes and regulations.

b. Establish offices or facilities that shall serve as reviewing or approving authorities of animal use proposals from subordinate activities and extramural facilities proposing research under contract or grant.

c. Provide members to JTWG as required.

d. Designate the appropriate office(s) within the DoD Component that shall perform the headquarters level administrative review of proposals requiring the use of non-human primates and shall serve as the office where exemptions under paragraph D.2. above may be approved.

e. Support, and as necessary, ensure the development of animal care and use training programs for researchers and members of the IACUC, and certification programs for all personnel involved in the care, use, and treatment of animals.

3. The Secretary of the Army shall:

a. As Executive Agent, develop and issue, in consultation with the other DoD Components, joint Service regulations to implement this Directive.

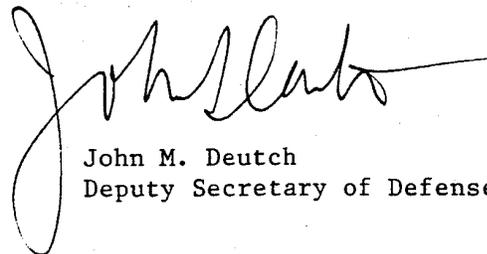
b. Designate the Commander, U.S. Army Veterinary Command/Director, DoD Veterinary Services Activity, a Field Operating Agency of the Army, Office of the Surgeon General who shall serve as a consultant to the Assistant Secretary of Defense for Health Affairs and the Director, Defense Research and Engineering for technical and professional matters related to this Directive.

F. EFFECTIVE DATE

This Directive is effective immediately.

Enclosures - 3

1. References
2. Definitions
3. Guidance Documents



John M. Deutch
Deputy Secretary of Defense

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- (e) National Institutes of Health (NIH) Publication No. 86-23, "Guide for the Care and Use of Laboratory Animals", United States Department of Health and Human Services, National Institutes of Health, Revised 1985.
- (f) Title 5, United States Code, Section 3109.

DEFINITION OF TERMS

1. Animal. - Any dog, cat, non-human primate, guinea pig, hamster, rabbit or any other live vertebrate animal, which is being used or is intended for use for research, training, testing, or experimentation purposes. For this Directive, it includes birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, training, testing or experimentation purposes. The term excludes animals used for ceremonial or recreational purposes, military working animals, and animals intended for use as livestock and poultry as food or fiber; or, livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.
2. Clinical Investigation. - All activities directed towards clinical research conducted principally within medical treatment facilities. The Clinical Investigations program is part of the Defense Health Program of the Assistant Secretary of Defense (Health Affairs) and is supported by Major Force Program 8 (MFP-8) funds.
3. Instructional Program. - All educational and training activities, except training of ceremonial and recreational animals and training associated with military working animals or survival skills training.
4. Research, Development, Test, and Evaluation. - All activities which form the RDT&E program of the Director, Defense Research and Engineering (DDR&E) and are supported by Major Force Program 6 (MFP-6) funds.
5. Alternatives. - Any system or method that covers one or more of the following: replacing or reducing the number of laboratory animals required for an investigation by computer simulation, cell culture techniques, etc; or, refining an existing procedure or technique to minimize the level of stress endured by the animal.
6. DoD Sponsored Programs. - All proposals or designs for animal experiments or demonstration in RDT&E, clinical investigation, or instructional programs conducted or funded by grant, award, loan, contract, or cooperative research and development agreement (CRADA).

**ADDITIONAL FEDERAL STATUTES, REGULATIONS,
AND GUIDELINES ON THE USE OF ANIMALS**

The following documents provide national standards and guidance for the protection, treatment and use of animals:

- a. **Animal Welfare Act** (Title 7, United States Code, Sections 2131-2158, as amended, and Title 9, Code of Federal Regulations, Parts 1-4, implementing rules and regulations). Administered by Regulatory Enforcement and Animal Care (REAC), Animal and Plant Health Inspection Service (APHIS) of the Department of Agriculture. Requires licensing of dealers, identification of animals, maintenance of records, submission of reports, establishment of an Institutional Animal Care and Use Committee (IACUC), and compliance with standards for the humane handling, care, treatment, and transportation of animals by dealers and research facilities.
- b. **Endangered Species Act of 1973** (Title 16, United States Code, Sections 1531-1543, as amended, and Title 50, Code of Federal Regulations, Parts 10-14 and 217-227, implementing rules and regulations). Provides a program under the U.S. Fish and Wildlife Service, Department of Interior, for conserving threatened and endangered species. Requires import/export permits, maintenance of records, and submission of reports on the care and handling of endangered, threatened, and conserved species.
- c. **Marine Mammal Protection Act** (Title 16, United States Code, Sections 1361-1384, as amended, and Title 50, Code of Federal Regulations, Parts 10-14 and 216-227, implementing rules and regulations). Provides a program under the Departments of Commerce (National Marine Fisheries Service) and Interior (U.S. Fish and Wildlife Service) for the protection of marine mammals and marine mammal products. Requires acquisition permits, maintenance of records, submission of reports, and inspections on the care and handling of marine mammals.
- d. **Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)** (TIAS 8249, as amended, and Title 50, Code of Federal Regulations, Part 23, implementing rules and regulations). CITES is a treaty involving 106 signatory nations administered in the United States by the Fish and Wildlife Service of the Department of the Interior. CITES regulates the import and export of imperiled species covered by the treaty but imposes no restrictions or control on interstate shipments.
- e. **Lacey Act** (Title 18, United States Code, Section 42, as

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amended, and Title 50, Code of Federal Regulations, Part 16 and Subpart B, implementing rules and regulations). A program under the U.S. Fish and Wildlife Service, Department of the Interior. Prohibits the importation of certain wild animals or their eggs if the Secretary of the Interior determines that they are injurious to humans, the interest of agriculture, or other specified national interests.

f. **Guide for the Care and Use of Laboratory Animals.** Public Health Service, National Institutes of Health, NIH Publication No. 86-23, Revised. Provides guidelines for institutional policies, husbandry, requirements, veterinary care, and physical plant requirements for programs involving the care and use of laboratory animals.

g. **Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.** Published by the Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 309 West Clark Street, Champaign, IL 61820, March 1988. Provides guidelines for the care and use of the major agricultural animal species in the United States in research and teaching.

APPENDIX B

DoD POLICY FOR COMPLIANCE WITH FEDERAL REGULATIONS AND DoD DIRECTIVES FOR THE CARE AND USE OF LABORATORY ANIMALS IN DoD-SPONSORED PROGRAMS



OFFICE OF THE SECRETARY OF DEFENSE

WASHINGTON, D.C. 20301

10 APR 1995

MEMORANDUM FOR ASSISTANT SECRETARY OF THE ARMY (M&RA)
ASSISTANT SECRETARY OF THE ARMY (RDA)
ASSISTANT SECRETARY OF THE NAVY (M&RA)
ASSISTANT SECRETARY OF THE NAVY (RDA)
ASSISTANT SECRETARY OF THE AIR FORCE
(MRAI&E)
ASSISTANT SECRETARY OF THE AIR FORCE (SAF/AQ)
PRESIDENT, UNIFORMED SERVICES UNIVERSITY OF THE
HEALTH SCIENCES
DIRECTOR, DEFENSE NUCLEAR AGENCY
DIRECTOR, ADVANCED RESEARCH PROJECTS AGENCY

SUBJECT: Department of Defense (DoD) Policy for Compliance with
Federal Regulations and DoD Directives for the Care and
Use of Laboratory Animals in DoD-Sponsored Programs

References:

(a) Title 7, United States Code, Sections 2131-2156,
The Laboratory Animal Welfare Act of 1966, PL 89-544,
as amended PL 94-279, 1976, and PL 99-198, 1985.

(b) Review of the Use of Animals in the Department of
Defense Medical Research Facilities, Inspector General
Department of Defense, February 1994.

(c) Review of the Use of Animals in Department of
Defense Contract Research Facilities, Inspector
General Department of Defense, August 1994.

Definition:

(a) Animal means any dog, cat, non-human primate, or
any other live vertebrate animal which is being used
or is intended for use for research, training, testing,
or experimentation purposes. For this Policy Guidance,
it includes birds, rats of the genus Rattus and mice of
the genus Mus bred for use in research, training,
testing or experimentation purposes. The term excludes
animals used for ceremonial or recreational purposes,
military working animals, and animals intended for use
as livestock and poultry as food or fiber; or,
livestock or poultry used or intended for use for
improving animal nutrition, breeding, management, or
production efficiency, or for improving the quality of
food or fiber.

(b) DoD-Sponsored programs means any study, proposal,
or design for animal experimentation or demonstration
in Research Development, Test, and Evaluation (RDT&E),
clinical investigation, or instructional program
conducted or funded by grant, award, loan, contract, or
cooperative research and development agreement (CRADA).

Reference (a) has been accepted by the Department of Defense (DoD) in the development of DoD Directives and policy guidance. References (b) and (c) contain recommendations which have been endorsed by the Department. The purpose of this policy memorandum is to implement the recommendations contained in references (b) and (c).

DoD components that utilize animals in DoD-supported programs shall be aware of the attached DoD Directive 3216.1, "Use of Laboratory Animals in DoD Programs," appended as attachment (1). It is currently pending signature and will supersede the current DoD Directive 3216.1 dated February 1, 1982. Additional policy guidance is as follows:

a) In DoD component facilities conducting animal-based programs, an alternate to the non-affiliated member of the Institutional Animal Care and Use Committee (IACUC) shall be designated for IACUCs having a single non-affiliated member. The non-affiliated member(s) or alternates must receive a minimum of eight hours training. At least four hours of the training shall address the regulatory responsibilities and proper techniques on animal protocol review processes. An additional minimum of four hours of training will address humane care and ethics issues dealing with animal use. All DoD Components conducting animal use programs as defined shall have training programs for non-affiliated IACUC members in place by 1 October 1995.

b) All DoD component facilities maintaining animals used in research, testing, or training shall apply for accreditation by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The Office of the Director, Environmental and Life Sciences, Pentagon Room 3D129, Washington, D.C. 20301-3030 is the central point of contact to maintain cognizance over the application or continuation of AAALAC accreditation. All DoD facilities shall furnish copies of AAALAC accreditation status to that office. Absence of accreditation shall be explained with a plan of action and milestones to obtain accreditation.

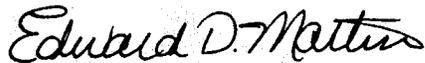
The following recommendations from the DoD Inspector General have been adopted as policy and shall be fully implemented by DoD Components which use animals in DoD-sponsored programs.

a) The DoD standard protocol format appended as attachment (2) shall be implemented by 1 October 1995. All intramural protocols involving animal use submitted after 1 October 1995 shall use the standard format. Extramural contractor proposal submissions need not use the standard format; however, the contractor shall provide all pertinent information contained in the standardized protocol format.

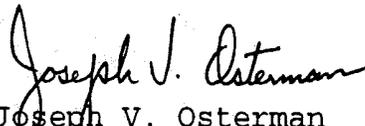
b) All DoD component facilities that utilize animals in research, testing and training shall implement the DoD standardized semi-annual program review checklist appended as attachment (3) immediately. Accompanying the checklist is a detailed outline of program review as contained in the NIH Guide for the Care and Use of Laboratory Animals. The Guide is the primary reference which is used by AAALAC in the accreditation process. The checklist shall be completed as a part of the semiannual IACUC program and facility review process. The semi-annual IACUC reports shall contain a copy of the checklist or indicate that the checklist was used as the basis of the program and facility review. A majority of members of the IACUC shall sign the report and include a statement indicating the presence or absence of minority opinions.

c) Commanders, and Directors of DoD component facilities shall support and, as necessary, develop animal care and use training programs for personnel associated with animal use programs, and encourage certification for all personnel involved in the care, use and treatment of laboratory animals.

As of 1 October 1995, DoD components shall report all animal-based protocols in the required format redacted for public release to the Defense Technical Information Center (DTIC). Selected fields of the DTIC report will be made accessible to the public through the INTERNET.



Edward D. Martin
Principal Deputy,
Assistant Secretary of
Defense (Health Affairs)



Joseph V. Osterman
Director, Environmental
and Life Sciences

Attachments:

- (1) Pending DoD Directive 3216.1
- (2) Standard Protocol Format
- (3) Standard Semi-annual Checklist

APPENDIX C

DoD STANDARD IACUC PROTOCOL FORMAT INSTRUCTIONS

ALL DOD ANIMAL USE PROTOCOLS MUST UTILIZE THIS DOD STANDARDIZED FORMAT. This protocol format only includes those requirements of the Animals Welfare Act, American Association for the Accreditation of Laboratory Animal Care, Federal Regulations, DoD Directives and DoD Policy relating to animal use. Any requirements that are specific to a given Service, Command, or locale (such as all budgeting information, local coordinating requirements, specific scientific review requirements etc.) should be added by each organization in front or behind this standardized format. Adding some information within the format is acceptable to meet local needs as long as the standard format is maintained. In other words, all of the labelled paragraphs and subparagraphs should remain in the same relative order with the added information being similar or complementary to the information requested. It is important to note that this standardized protocol format does not in any way prohibit local organizations from using any (or all) of their current animal use protocol. It does mandate that all of the information required in this DoD standardized format be answered as a part of the organization's animal use protocol in the order listed in this format.

THIS DOCUMENT IS INTENDED TO BE AN AID IN THE PREPARATION OF A DOD ANIMAL USE PROPOSAL. IT IS A COMPANION DOCUMENT TO AN IDENTICAL PROTOCOL FORMAT OR TEMPLATE THAT DOES NOT HAVE THE WRITTEN EXPLANATION FOR INDIVIDUAL PARAGRAPHS. THEY ARE DESIGNED TO BE USED ON A WORD PROCESSING PROGRAM, i.e., WordPerfect, WordStar, MicrosoftWord, WordPerfect for Macintosh, etc., SO THAT YOU ARE NOT LIMITED BY THE SPACE PROVIDED, AND SUGGESTED CHANGES OR MODIFICATIONS CAN BE QUICKLY AND EASILY MADE. USING A WORD PROCESSOR MAKES THIS FORMAT A "FILL-IN-THE-BLANKS" EXERCISE. THE EXPLANATIONS OR INSTRUCTIONS MAY BE BLOCKED OUT AND DELETED IF IT IS MORE CONVENIENT TO USE THIS FORM RATHER THAN THE OUTLINE AVAILABLE WITHOUT THE EXPLANATIONS. SPECIFIC RESPONSES REQUESTED IN THE FORMAT ARE A RESULT OF THE REQUIREMENTS OF THE ANIMAL WELFARE ACT (AWA), DOD REGULATIONS, OR ANIMAL WELFARE GUIDELINES. EACH PARAGRAPH SHOULD HAVE A RESPONSE. PORTIONS OF THE PROTOCOL FORMAT THAT ARE NOT APPLICABLE TO YOUR PARTICULAR PROTOCOL, i.e., NO SURGERY OR NO PROLONGED RESTRAINT, SHOULD BE MARKED N\A. IF SOPs OR OTHER DOCUMENTS ARE READILY AVAILABLE TO THE IACUC, THEY MAY BE REFERENCED TO ASSIST IN THE DESCRIPTION OF SPECIFIC PROCEDURES. IT IS CRITICAL THAT ONLY ANIMAL STUDIES OR PROCEDURES DOCUMENTED IN AN APPROVED PROTOCOL ARE PERFORMED IN THE ORGANIZATION. ADDITIONALLY, P.I.s OR OTHER ANIMAL USERS SHOULD KEEP ACCURATE EXPERIMENTAL RECORDS, AND BE ABLE TO PROVIDE AN AUDIT TRAIL OF THEIR ANIMAL EXPENDITURES AND USE THAT CORRELATES TO APPROVED PROTOCOLS.

PROTOCOL COVER SHEET: Requires a minimum of three signatures to include: the Primary Investigator, the individual responsible for scientific review and the Attending Veterinarian. In addition, the signature from the individual performing the statistical review on this cover sheet is recommended. If no signature block is present for a person who does the statistical review, then the following statement must be present on the protocol cover sheet. "A person knowledgeable in statistics has reviewed the experimental design." This Protocol Cover Sheet can also hold any additional information deemed necessary by the organization (Co- investigators, Department/Division Chief, Coordinating Departments, IACUC Chair, Biosafety Review etc.)

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

(Signature Required)

(Principal Investigator)

SCIENTIFIC REVIEW: Signature verifies that this proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice. (No response is required to the title paragraph of this section)

(Signature Required)

(Research Unit Chief/Directors signature)

ATTENDING/CONSULTING VETERINARIAN: (Example) The attending/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/veterinary medicine department has assisted with coordination for veterinary support to the protocol. (No response is required to the title paragraph of this section)

(Signature Required)

(Attending/Consulting Veterinarian)

STATISTICAL REVIEW: A person knowledgeable in statistics has reviewed the experimental design. (No response is required to the title paragraph of this section) (Inclusion of Signature Block is Recommended, but Optional)

(Statistician)

OTHERS: You may wish to add specific additional offices or signature blocks for individuals responsible for coordination or compliance issues pertinent to your facility or operation. (i.e. Co- investigators, Coordinating Departments, IACUC Chair, Biosafety Review etc.)

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

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

II. BACKGROUND:

A. Background: This should 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 should be provided. Unnecessary duplication of effort should be strictly avoided.

B. Literature Search: This search must be performed to prevent unnecessary duplication of previous experiments. A search of Federal Research in Progress (FEDRIP) and DTIC databases or their equivalent is required for DOD funded research. An additional search of the scientific literature (MEDLINE, GRATEFUL MED, MEDLARS, AWIC, etc.) is highly recommended.

1. 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(s).

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

IV. MILITARY RELEVANCE: With regards to military needs and mission requirements, this paragraph should provide a brief and succinct military justification for the research. 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." This section should succinctly outline the formal scientific plan and direction for experimentation. If several experiments or sequential studies

are to be included in the protocol, description of the experimental design for each separate experiment should be contained 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. If too few animals are requested and statistical significance is not achieved, the animals will have been misused. Be certain to include animals necessary for controls or technique development, etc. If the design is complex, a summary table or flow chart showing the distribution of animals by experimental group should be included. **The total number of animals required for the study is listed in section V.B.4. It is critical that reviewers of this protocol are able to follow your 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: (etc.)

B. Laboratory Animals Required and Justification:

1. **Non-animal Alternatives Considered:** Were alternatives to animal use considered? **No study using animals should be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means, i.e., computer modeling, cell cultures, etc.**

2. **Animal Model and Species Justification:** It is important that you adequately justify that animals are necessary for attainment of the research/training objectives. Moreover, justify the selection of this particular animal model. Investigators should use the least sentient species that will permit the attainment of research objectives. Why was this particular animal chosen? Were there other animal models considered that are lower on the phylogenetic scale (e.g., mice instead of rabbits)? Is 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, please use proper terminology.

c. Source/Vendor: Provide a preferred source for the animals. Procurement of animals from non-USDA licensed sources requires an exception to policy. Enter the source/vendors USDA license number if available.

d. Age:

e. Weight:

f. Sex:

g. Special Considerations: Specialized requirements for the research animals should be reflected here, i.e., SIV or herpes antibody free, Pasteurella free, etc.

h. Other:

4. Total Number of Animals Required:

(a) mice	320
(b) guinea pigs	175

All that is required in this section is the total number of animals to be used on the study. The number requested here should match exactly those described in para V. A., Experimental Design & General Procedures in the MATERIALS AND METHODS section. Keep in mind the number requested should be the minimum numbers necessary to complete the study, but must be sufficient to yield meaningful results. If too few animals are requested and statistical significance is not achieved, the animals will have been misused. Be certain to include animals necessary for controls or technique development, etc. 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.

5. Refinement, Reduction, Replacement: The DoD is often required to provide specific examples of its alternatives initiatives. Does this protocol have any provisions that would qualify it to be identified as one that refines, reduces or replaces (3 R's) the use of animals? For example, does your study use statistical tests that require fewer animals, i.e., a modified LD50 test like Thompson & Weil, or are you using cell cultures, computer modeling or any other technique that will influence the numbers of animals required? Are you using animals lower on the phylogenetic scale? Please provide a short description of the features that you feel qualify the study as one that employs one of the "3 R's," or give a negative reply. No response is needed under the title paragraph of this section.

a. Refinement: The use of analgesia, or the use of remote telemetry to increase the quality and quantity of data

gathered or adjusted early endpoint for the animals are examples of refinements.

b. Reduction: Use of shared control groups, preliminary screening in non-animal systems or innovative statistical packages are examples of reductions.

c. Replacement: Non-animal systems that eliminate the use of animals are examples of replacement.

C. Technical Methods: These should 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, 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.** Respond N\A if the animals will experience "no pain or distress."

a. USDA (Form 18-3) Pain category:

This information is reported by the organization to the USDA on USDA Form VS 18-23. **The P.I. or primary user should 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, i.e., 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 should add up to the number and percent of animals requested for the entire protocol in para V.B.4.**

(1) No Pain _____ (#) _____ % (Column C)

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.

**(2) Alleviated Pain _____ (#) _____ %
(Column D)**

Procedures wherein 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 would be examples for this category.

(3) Unalleviated Pain or Distress

_____ (#) _____ % (Column E)

Procedures where alleviation of pain or distress are contraindicated for some justifiable reason such as would confound the experimental results if drugs relieving pain were administered. Detailed justification for putting animals into this category is required below in para V.C.1.d.

b. Pain Alleviation: The attending veterinarian should be able to provide assistance in completing this section of the proposal.

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

(2) Paralytics: No use of paralytic agents without anesthesia is allowed unless scientifically justified by the P.I. and approved by the IACUC.

c. Alternatives to Painful Procedures:

(1) Source(s) Searched: e.g., AWIC, AGRICOLA, CAAT, MEDLINE, etc.

(2) Date of Search:

(3) Key Words of Search: e.g. Pain, surgery,

(4) Results of Search: 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." It is a requirement to perform the alternatives literature search and painful procedure justification even when animals are placed in the alleviated pain category (column D).

d. Painful Procedure Justification: Procedures causing more than transient or slight pain that are unalleviated, 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 para V.C.1. **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 it here.**

2. Prolonged Restraint: Describe and justify in detail any prolonged restraint (greater than twelve hours) intended for use during the study, e.g., 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 be restraining the animals, and for how long?

3. Surgery: Major operative procedures on non-rodent species, i.e., rabbits, monkeys, etc., should be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions. Non-major operative procedures & all rodent surgery do not require a dedicated facility, but must be performed using aseptic technique, i.e., surgical gloves, mask, 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. 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.

c. Location: Give the location\room # for the proposed surgical procedure.

d. Multiple Survival Surgery Procedures: If multiple major operative procedures on the same animal are intended, they must be adequately justified for scientific reasons by the P.I. in writing.

(1) **Procedures:**

(2) **Scientific Justification:**

4. **Animal Manipulations:** Any injections, sampling procedures, or other manipulations of the animals necessary for the execution of the study must be described if not listed in section V. List needle sizes, routes of injection or withdrawal and anatomical location, e.g. 21 ga needle, SQ, IM, femoral vein, jugular vein etc., or the proposed method so that a reasonable evaluation of the appropriateness of the procedure can be made. You may furnish the committee a reference or SOP to document a particular procedure in lieu of a detailed description. You may wish to rearrange the subparagraphs of this section to suit your protocol. No response is needed under the title paragraph of this section.

a. **Injections:** There is no need to duplicate specific information already provided in section V.C.1.b., the Pain Alleviation, anesthesia/analgesia section of the proposal.

b. **Biosamples:** Cerebral taps, blood sampling, etc. List amounts taken and method for sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

c. **Animal Identification:** Microchip, tattoo, 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 this study.

e. **Other procedures:** EKG's, radiology, aerosol exposure, etc.

5. **Adjuvants:** List any adjuvants and your plan for their use. Provide dosages & route.

6. **Study Endpoint:** What is the projected end point or termination of the study for the animals? Is death, euthanasia, or recovery expected; and what is the specific plan for determining when the animal experimentation phase will be stopped? You should 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. **You must 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 who will perform the procedure. The 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 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.

D. Veterinary Care: Attending veterinary care of lab animals receives particular emphasis in the AWA. The attending veterinarian of your 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. Describe husbandry or refer to SOP. If known, list the location the animals will be routinely housed and the length of housing requirement. Personnel in the animal care unit should be able assist P.I.s in the preparation of the protocol sections dealing with animal care issues.

a. Study Room: If stay exceeds 12 hours.

b. Special Husbandry Provisions: Micro-isolators, metabolic cages, etc.

2. Attending Veterinary Care: Will the animals be observed daily or more frequently, and by whom? What is the plan if the animal becomes ill or debilitated during the study and requires supportive therapy? Will the animal be euthanized if it becomes critically ill or comatose, and by whom (study endpoint adjustment)? Justification for not providing supportive care for clinically ill animals is necessary.

3. Enrichment Strategy: Written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates must be provided.

a. Dogs: Do you have 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. If yes, justify.

b. Nonhuman Primates: Do you have any reason to prohibit environmental enrichment or enhancement strategies that might be implemented by the animal care unit to comply with federal welfare regulations. If yes, justify.

E. Data Analysis: List the statistical test(s) planned or the strategy intended to evaluate the data.

F. Investigator & 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 that procedure. **This is NOT 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 your attending veterinarian for assistance with this requirement.

VI. Biohazard/Safety: Provide a list of any potential biohazards associated with this proposal, e.g., 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.

(Start new page here)

VII. ASSURANCES: The law specifically requires several written assurances from the P.I. 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."

(This section will state) As the Primary Investigator on this protocol I acknowledge my responsibilities and provide assurances for the following:

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 an individual who is qualified 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 regarding 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 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.

(Signature Required)

(Primary Investigator)

G. Painful Procedures: (Include only if conducting research that will cause more than slight or momentary pain or distress (Column D or E by USDA classification) the following statement must follow.) **I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved 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.

(Signature Required)

(Primary Investigator)

VIII. Enclosures: (Available for the attachment of the results of any literature searches, SOPs, references, or other documents pertinent to the protocol that you may wish to include. Local IACUC's should determine specific items to be included here.)

A. Literature Searches: DTIC, FEDRIP, MEDLINE, AGRICOLA, etc.

B. Pathology Addendum: Optional information

C. Pain Scoring Guidelines:

D. Adjuvant Policy:

PROTOCOL COVER SHEET

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

(Signature Required)

(Principal Investigator)

SCIENTIFIC REVIEW:

(Signature Required)

(Research Unit Chief/Directors signature)

ATTENDING/CONSULTING VETERINARIAN:

(Signature Required)

(Attending/Consulting Veterinarian)

STATISTICAL REVIEW: A person knowledgeable in statistics has reviewed the experimental design. (No response is required to the title paragraph of this section) (Inclusion of Signature Block is Recommended, but Optional)

(Statistician)

***OTHERS:** You may wish to add specific additional offices or signature blocks for individuals responsible for coordination or compliance issues pertinent to your facility or operation. (i.e. Co-investigators, Coordinating Departments, IACUC Chair, Biosafety Review etc.)

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

- I. **NON-TECHNICAL SYNOPSIS:**
- II. **BACKGROUND:**
 - A. **Background:**
 - B. **Literature Search:**
 - 1. **Literature Source(s) Searched:**
 - 2. **Date and Number of Search:**
 - 3. **Key Words of Search:**
 - 4. **Results of Search:**
- III. **OBJECTIVE\HYPOTHESIS:**
- IV. **MILITARY RELEVANCE:**
- V. **MATERIALS AND METHODS:**
 - A. **Experimental Design and General Procedures:**

B. Laboratory Animals Required and Justification:

- 1. Non-animal Alternatives Considered:**
- 2. Animal Model and Species Justification:**
- 3. Laboratory Animals:**
 - a. Genus & Species:**
 - b. Strain/Stock:**
 - c. Source/Vendor:**
 - d. Age:**
 - e. Weight:**
 - f. Sex:**
 - g. Special Considerations:**
 - h. Other:**
- 4. Total Number of Animals Required:**
- 5. Refinement, Reduction, Replacement:**
 - a. Refinement:**
 - b. Reduction:**
 - c. Replacement:**

C. Technical Methods:

- 1. Pain:**
 - a. USDA (Form 18-3) Pain category:**
 - (1) No Pain _____ (#) _____% (Column C)
 - (2) Alleviated Pain _____ (#) _____% (Column D)
 - (3) Unalleviated Pain or Distress
_____ (#) _____% (Column E)
 - b. Pain Alleviation:**
 - (1) Anesthesia/Analgesia/Tranquilization:
 - (2) Paralytics:
 - c. Alternatives to Painful Procedures:**
 - (1) Source(s) Searched:
 - (2) Date of Search:
 - (3) Key Words of Search:
 - (4) Results of Search:
 - d. Painful Procedure Justification:**
- 2. Prolonged Restraint:**
- 3. Surgery:**
 - a. Procedure:**
 - b. Pre- and Postoperative Provisions:**
 - c. Location:**
 - d. Multiple Survival Surgery Procedures:**
 - (1) Procedures:
 - (2) Scientific Justification:
- 4. Animal Manipulations:**
 - a. Injections:**
 - b. Biosamples:**
 - c. Animal Identification:**
 - d. Behavioral Studies:**
 - e. Other procedures:**
- 5. Adjuvants:**
- 6. Study Endpoint:**

- 7. Euthanasia:
 - D. Veterinary Care:
 - 1. Husbandry Considerations:
 - a. Study Room:
 - b. Special Husbandry Provisions:
 - 2. Attending Veterinary Care:
 - 3. Enrichment Strategy:
 - a. Dogs:
 - b. Nonhuman Primates:
 - E. Data Analysis:
 - F. Investigator & Technician Qualifications/Training:
- VI. Biohazard/Safety:

(Start new page here)

VII. ASSURANCES: As the Primary Investigator on this protocol I provide the following assurances:

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 an individual who is qualified 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 regarding 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 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.

(Signature Required)

(Primary Investigator)

G. Painful Procedures: (Include above if conducting research that will cause more than slight or momentary pain or distress (Column D or E by USDA classification) the following statement must follow.) **I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved 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.

(Signature Required)

(Primary Investigator)

VIII. Enclosures: (Available for the attachment of the results of any literature searches, SOPs, references, or other documents pertinent to the protocol that you may wish to include. Local IACUC's should determine specific items to be included here.)

- A. Literature Searches: FEDRIP, DTIC, MEDLINE, AGRICOLA, etc.
- B. Pathology Addendum: Optional information
- C. Pain Scoring Guidelines:
- D. Adjuvant Policy:

APPENDIX D

DoD SEMIANNUAL PROGRAM REVIEW AND FACILITY INSPECTION CHECKLIST

DOD SEMIANNUAL PROGRAM REVIEW/FACILITY INSPECTION CHECKLIST-MANDATORY

Completion of this one-page checklist by the IACUC during the semi-annual program review and facility inspection is mandatory.

ORGANIZATION: _____ **DATE OF REVIEW:** _____

EVALUATION VIA CATEGORY	S	M	U	NA	EVALUATION VIA CATEGORY	S	M	U	NA
AAALAC History					Identification Records				
Administrative Commitment					Emergency, Weekend & Holiday Care				
Administrative Organization					Adequate Veterinary Care				
Institutional Policies					Preventive Medicine				
Animal Care & Use Committee					Animal Procurement				
Protocol Review Procedures					Quarantine Isolation				
Personnel Qualifications					Control of Animal Disease				
Personnel Hygiene					Diagnostic Resource				
Occupational Health Program					Anesthesia & Analgesia				
Animal Restraint					Surgery & Postsurgical Care				
Multiple Major Surgeries					Euthanasia				
Animal Husbandry					Physical Plan Arrangement/Cond.				
Housing/Caging & Pens					Support Areas				
Social Enrichment					Cage Sanitation Fac.				
Activity/Exercise					Storage Facilities				
Food/Water/Bedding					Surgery Facilities				
Sanitation					Animal Rooms				
Waste Disposal Methods					HVAC				
Vermin Control					Emergency Power				
Farm Facilities					Animal Use Laboratories				

KEY: S = Satisfactory; M = Minor Deficiency; U = Unsatisfactory/Major deficiency; NA = Not Applicable

USE OF CHECKLIST IN PROGRAM EVALUATION-- Completion of this one page checklist is mandatory. Any area that has minor or Major/Unsatisfactory deficiencies should be further explained on a separate page(s). Moreover, the listing of the minor or major deficiency should also include a plan of action for correction of the deficiency.

DETAILED OUTLINE OF CHECKLIST-- Utilization of this outline is optional. Attached is a detailed outline which follows this checklist. The outline includes most additional DoD requirements and is very similar to the program description outline used by organizations applying for AAALAC accreditation. This outline or one devised by your IACUC can be used to augment your semi-annual program reviews.

USE OF ROOM INSPECTION FORM-- Utilization of attached form is optional. The use of this form or one developed by your organization may be useful in augmenting your semi-annual program review.

MINORITY OPINIONS-- Utilization of attached form is optional. All minority opinions must be included in the IACUC report. In addition it is mandatory that a majority of IACUC members sign the semi-annual report.

There were / were not (circle one) minority opinions in this semi-annual review.

-OPTIONAL-

DETAILED OUTLINE OF CHECKLIST-- Utilization of this outline is optional. Attached is a detailed outline which follows this checklist. The outline includes most additional DoD requirements and is very similar to the program description outline used by organizations applying for AAALAC accreditation. This outline or one devised by your IACUC can be used to augment your semiannual program reviews.

A. General Comments AAALAC history, administrative commitment, administrative organization,

B. Institutional Policies

1. Monitoring the Care and Use of Animals

- a. Institutional Animal Care and Use Committee
 - 1) Composition- New DoD Directive states the minimum number for IACUC membership is 5. New DoD policy states requires those IACUCS with only one non-affiliated member the IACUC to also appoint an additional alternate non-affiliated member. New DoD policy states specific training requirements for non-affiliated IACUC members (8 hours).
 - 2) Protocol review procedures- New DoD Directive and policies require use of DoD standard protocol format. New requirements include documentation of literature searches for DTIC, FEDRIP and other searches as required.
 - 3) Review of programs for Care and Use of Animals- New DoD policy encourages Commanders/Directors/CEO's of DoD laboratories to invest in training at all levels for those that use animals.

b. USDA Report

2. Veterinary Care

- a. Intensity -
- b. Responsibilities of the Veterinarian(s) -
- c. Involvement in monitoring the care of animals -
- d. Involvement in monitoring use of animals -

3. Personnel Qualifications

- a. Animal resource Professional/Management/ Supervisory Personnel -
- b. Animal Care Personnel -
- c. Research Staff -
- d. Use of Hazardous Agents -

4. Personnel Hygiene

- a. Work clothing provided -
- b. Laundering of work clothing -
- c. Shower and change facilities -
- d. Eating, drinking, and smoking policies -
- e. Eating, drinking, and smoking facilities -

5. Occupational Health and Safety Program

- a. Content of program -
- b. Program oversight -
- c. Participation by staff -
- d. Training on zoonosis and personal hygiene -

6. Experimentation involving Hazardous Agents

7. Animal Restraint -

8. Multiple Major Surgical Procedures -

C. Laboratory Animal Husbandry

1. Housing

- a. Caging and pens -

DoD Semiannual Program Review/Facility Inspection

- b. Social enrichment -
- c. Activity/exercise -
- d. Micro- & Macroenvironments -
- 2. **Food**
 - a. Type -
 - b. Vendor quality control -
 - c. Storage -
 - d. Type of feeders -
 - e. Institutional quality control -
- 3. **Bedding**
 - a. Type -
 - b. Appropriateness for how used -
 - c. Storage facilities -
 - d. Quality control -
- 4. **Water**
 - a. Source - Satisfactory.
 - b. Treatment - Satisfactory.
 - c. Quality control procedures -
- 5. **Sanitation**
 - a. Cage & pan litter changing -
 - b. Portable cage sanitation
 - 1) Frequency -
 - 2) Procedures and agents -
 - 3) Monitoring and effectiveness -
 - c. Pens, Stalls, etc. -
 - d. Sanitation of feeding implements -
 - e. Watering Implements
 - 1) Water Bottles -
 - 2) Automatic watering system -
 - f. Sanitation of transport cages and vehicles -
 - g. Room sanitation -
 - h. Waste disposal methods -
 - i. Vermin control -
- 6. **Animal Identification**
 - a. Methods for identification of each species -
 - b. Information of cage cards -
 - c. Individual animal records -
- 7. **Provisions for Emergency, Weekend and Holiday Care**
 - a. Qualifications of individuals providing care -
 - b. Procedures performed -
 - c. Monitoring of environmental systems -

D. Veterinary Care

1. Preventive Medicine

- a. Animal procurement -
- b. Quarantine, Stabilization and Isolation -
 - 1) Receiving and initial evaluation procedures -
 - 2) Quarantine facilities
 - a) For random source animals -
 - b) For purpose bred animals -

- 3) Quarantine procedures -
- c. Separation by species, source and health status -
- 2. **Surveillance, Diagnosis, Treatment, and Control of Animal Disease**
 - a. Program
 - 1) Daily observation of animals -
 - 2) Procedures for providing veterinary care -
 - 3) Medical Records maintenance procedures -
 - 4) Preventive medicine program for each species -
 - 5) Animal Health monitoring -
 - b. Diagnostic Resources
 - 1) Clinical Laboratory -
 - 2) Necropsy/histology -
 - 3) Radiology -
 - 4) Use of available diagnostic resources including commercial laboratories -
- 3. **Anesthesia and Analgesia**
 - a. Agents used for each species -
 - b. Guidelines provided by the Veterinarian -
 - c. Monitoring the use of A & A -
 - d. Training and experience of personnel who perform anesthesia -
 - e. Safety procedures for use of explosive/flammable agents -
 - f. Waste anesthetic gas scavenging -
- 4. **Survival Surgery and Postsurgical Care**
 - a. Non-rodent mammalian species
 - 1) Professional supervision -
 - 2) Qualifications of persons performing the surgery -
 - 3) Qualifications of surgical technicians -
 - 4) Aseptic Techniques -
 - 5) Postoperative care -
 - 6) Maintenance of PO care records -
 - b. Rodent species - use of cap, mask, surgical scrub, sterilized instruments used, hair clipped, .
 - c. Non-survival surgeries -

E. Physical Plant

- 1. **Overview of General Arrangement and Condition of Facility**
- 2. **Support Areas**
 - a. Clean cage storage -
 - b. Storage Areas -
 - c. Waste disposal facilities -
 - d. Lounge area for animal care personnel -
 - e. Administrative space -
 - f. Cage sanitation facilities -
 - 1) Interior surfaces -
 - 2) Sanitation equipment -
 - 3) Environmental conditions for personnel -
 - g. Surgery facilities
 - 1) Areas for
 - a) Surgery -
 - b) Animal preparation -
 - c) Dressing rooms -
 - d) Surgeon preparation -
 - e) Postoperative care -

DoD Semiannual Program Review/Facility Inspection

3. Animal Rooms

- a. Interior surfaces -
- b. Lighting - Satisfactory.
- c. HVAC -

4. Other Features

- a. Emergency power -
- b. Environmental monitoring
 - 1) Animal rooms air flow -
 - 2) Relative air pressures -
 - 3) Temperature -
 - 4) Humidity -
- c. Security -

5. Miscellaneous Animal Care and Use Equipment

F. Special Considerations

- 1. Genetics and Nomenclature -
- 2. Facilities and Procedures for Animal Research Involving Hazardous Agents -
- 3. Farm Animals -

G. Study Areas Visited -

H. Laboratories Visited -

DoD Semiannual Program Review/Facility Inspection

-OPTIONAL-

USE OF ROOM INSPECTION FORM--Utilization of attached form is optional. The use of this form or one developed by your organization may be useful in augmenting your semi-annual program review.

Building _____

=====

ROOM _____	Animal Holding Area	Lab	Other
-------------------	---------------------	-----	-------

=====

ROOM _____	Animal Holding Area	Lab	Other
-------------------	---------------------	-----	-------

=====

ROOM _____	Animal Holding Area	Lab	Other
-------------------	---------------------	-----	-------

=====

ROOM _____	Animal Holding Area	Lab	Other
-------------------	---------------------	-----	-------

=====

GENERAL COMMENTS:

APPENDIX E

U.S. GOVERNMENT PRINCIPLES FOR ANIMAL USE

U.S. Government Principles for Animal Use

Interagency Research Animal Committee's

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible institutional official shall ensure that these principles are adhered to:

- I. The transportation, care and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.¹
- II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
- III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.
- IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
- V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
- VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
- VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
- VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their inservice training, including the proper and humane care and use of laboratory animals.
- IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal research committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

¹ For guidance throughout these Principles the reader is referred to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

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APPENDIX F

BENEFITS OF DoD INTRAMURAL AND TRAINING PROGRAMS THAT USE ANIMALS

BENEFITS OF DoD INTRAMURAL AND TRAINING PROGRAMS THAT USE ANIMALS

Alternatives to Animal Research, Breeding Programs (A1, A2, B)

- Specific pathogen-free nonhuman primate colonies
- Laboratory technicians properly trained in animal handling and protocol procedures
- Development of a definitive and safe anesthetic regimen for chinchillas used in biomedical research

Clinical Medicine (C1)

- Study of the use of vasopressors in spinal shock/trauma
- Assessment of a new imaging agent (“Acutect”) to detect atrial thrombus formation and cardiac injury due to secondary pulmonary emboli
- Research on the development of a HIV vaccine
- Validation of a treatment regimen for osteoarthritis
- Development of a reliable and nonsurgical method (auditory brainstem response) for determining hearing measurements
- Additional insight into mechanism of cellular damage in muscular dystrophy
- Development of a new rat model of hypertension associated with type II diabetes
- Development of a more effective and rapid method of restoring body temperature to victims of hypothermia
- Better understanding of the development, diagnosis, and treatment of colon carcinomas
- Expansion of basic science knowledge in leukocyte physiology
- Greater understanding of the effects of hyperbaric oxygen on focal brain contusions
- Efficacy testing of a new fibrin sealant bandage to deliver high dose chemotherapy to locally advanced prostate cancer
- Research leading to understanding the etiology of schizophrenia and therapeutic approaches for civilian and military patients
- Research on the effects of a combined-treatment approach to repair chronic spinal cord injuries
- Information on gender differences in nicotine’s behavioral and psychological effects
- Increased knowledge of pharmacological treatments and prevention strategies for neuropsychiatric disorders such as Post Traumatic Stress Disorder in military and civilian victims

Clinical Surgery (C2)

- Four Investigational New Drug Applications awarded by the FDA (1 for phase 1 study of anti-CD154 in human volunteers, 2 for phase 2 trials with anti-CD154 in islet and kidney transplantation, and 1 for phase 1/2 study of anti-B7 antibodies in human renal transplantation) and transitioned into ongoing clinical trials
- Identification of a potential enzymatic, nonsurgical method of ear deformity recontouring
- Design of an invasive carcinoma surgical model to evaluate chemotherapeutic agent using fibrin adhesive
- Research and testing of a corneal implanted optical device to permit limited vision in severe cataract patients
- Studies of a blood substitute to be used in treating hemorrhagic shock following trauma with brain injury
- Provides military physicians with the opportunity to develop and perform surgical research
- Development of a more effective and efficient methodology for treating “empty eye socket” situations in growing children
- Better understanding of appropriate treatment of blood loss shock in the presence of traumatic brain injury using plasma replacements
- Research on skin transplants

Infectious Diseases (M2)

- Identification of two highly effective dengue vaccines
- Development of rapid diagnostic tests to identify caries
- Development of arboviral diagnostic assays for diagnosing dengue, Japanese encephalitis and Chikungunya elicit
- Determine that Shiga Toxin (STX) and other STX family members are potential biological warfare/terrorist threats
- Patent awarded: Ralls, S.A.; Rapid Immunoassay for Cariogenic Bacteria, U.S. Patent No. 6,015,681
- Development of an ELISA standard to measure the mucosal immune response to specific antigens
- Determine cause of up-regulation in apoptotic cells with neuronal morphology
- Testing of GMP Shigella vaccine products for immunogenicity, safety, and efficacy
- Studies and experiments addressing issues in infectious diseases such as malaria, HIV, and diarrheal disease, scrub typhus; ebola, gonorrhoeae

Medical Chemical Defense (M3)

- Maintain control of seizure activity with the use of advanced anticonvulsant treatments
- Discovered that doses of midazolam are efficacious against status epilepticus seizures
- Research on the mechanisms of action and physiological reactions of chemical agents
- Development of a Decision Tree Network for active topical skin protectants consisting of three testing modules that include in vitro, in vivo, and advanced testing

Medical Biological Defense (M4)

- Development of monoclonal antibodies specific to biological/chemical agent stimulants, environmental contaminants and biological toxins
- Development of a model to test the ability of Brucella vaccines to protect against infection following respiratory exposure to *Brucella melitensis*
- Demonstrated and characterized the development of bronchopneumonia, enanthema, exanthema, and consistent monocytosis
- Evaluate early stages of *Bacillus anthracis* spore infection
- Identification of attenuated vaccine candidates for Western Equine Encephalitis and Venezuelan Equine Encephalitis-IE viruses

Human Systems Technology (M5)

- Better understanding of treatment and prevention of “altitude sickness.”
- Production of recombinant and monoclonal antibodies for the development of rapid diagnostic/detection assays
- Development of a rat model to evaluate vascular permeability
- Laser studies permitted the establishment of exposure guidelines for both the military and private sectors
- Assessment of potential hazards and health risks of pulsed microwave radiation, in order to provide for safe electromagnetic environment for military personnel and define safe operation limits for irradiating military equipment
- Research on the effects of single versus multiple subthreshold blast overpressure exposures to lungs, heart, brain, kidney, liver, and gastrointestinal tract

Combat Casualty Care (M6)

- Establishment of a model of combined traumatic brain injury and hemorrhagic hypotension
- Research on the mechanism of mucus genes response to smoke inhalation
- Enhancement of the ability to control lethal hemorrhagic shock with the development of new hemostatic dressings and pharmacologic agents

- Providing surgeons with a real-time imaging tool to visualize thermal injury depth
- Research on resuscitation fluids and documentation of their benefits and side effects
- Investigation of potential treatment modalities for the stabilization of battlefield casualties at high risk of early death to profound hemorrhage and reduction in circulation

Ionizing Radiation (M7)

- Identification of protection against and treatment of radiation injury

Other Medical RDT&E (M8)

- Development of cleanup levels for toxins in soil and water
- Research on the mechanisms of human chronic fatigue syndrome
- Quantification of munitions compounds wildlife toxicity

Physical Protection (N1)

- Updating of the national and international laser safety standards

Other Non-Medical RDT&E (N4)

- Determination of the requirements, capabilities, and limitations of marine mammals use in operational Fleet Marine Mammal Systems
- Research on the bio-physical properties of the dolphin sonar capabilities and bio-mechanics
- Identification of environmental and human health risks factors
- Toxicological hazard evaluation of chemical threats
- Development of biomonitoring systems to evaluate source water quality

Training, Education, and/or Instruction of Personnel (T1)

- Increased medical readiness of assigned personnel by refining technical skills and surgical proficiency
- Training physicians in surgical techniques such as cardiovascular surgery, pediatric microsurgery, emergency surgery, obstetrical surgery, vascular and microvascular surgery
- Compliance with 9 CFR (the Animal Welfare Act regulations) where in research personnel are adequately trained and certified to perform animal procedures under controlled conditions prior to working on other approved protocols
- Training in life-saving measures for use in both combat and non-combat situations for health care providers

APPENDIX G

**DoD ANIMAL RESEARCH PUBLICATIONS BY
RESEARCH CATEGORY**

DoD ANIMAL RESEARCH PUBLICATIONS BY RESEARCH CATEGORY

JOURNALS

Animal Use Adjuncts/Alternatives (A1, A2)

Comparative Medicine
Toxicologic Pathology
Toxicological Sciences

Clinical Medicine (C1)

Journal of Neuroscience
Brain Research
Cell and Molecular Biology
Clinical and Experimental Hypertension
Drug Metabolism and Distribution
Gastroenterology
Journal of Endotoxin Research
Journal of Comparative Neurology
Journal of Endodontics
Journal of Immunologic Methods
Journal of Immunology
Journal of Infectious Diseases
Journal of Neuroscience
Journal of Periodontology
Journal of Pharmacology and Experimental
Therapeutics
Journal of the American Society of Nephrology
Laboratory Animal Science
Lasers in Surgery and Medicine
Muscle and Nerve
Neurology Report
Neurotrophic Factors
Pediatric Research
Proceedings of the National Academy of Sciences

Clinical Surgery (C2)

Circulation
Digestive Surgery
International Journal of Pediatric Otorhinolaryngology
International Journal of Radiation Oncology
Journal of American College of Surgery
Journal of the American College of Cardiology
Journal of Urology
Ophthalmic Plastic and Reconstructive Surgery
Vascular Surgery

Infectious Disease (M2)

Advanced Drug Delivery Reviews
American Journal of Tropical Medicine and Hygiene

Anatomy and Embryology
Arch. of Virology
Immunology Letters
Immunology
Immunology Today
Infection and Immunity
International Journal of Acarology
Journal of Biological Chemistry
Journal of Clinical Microbiology
Journal of General Virology
Journal of Immunological Methods
Journal of Immunology
Journal of Infectious Diseases
Journal of Medical Entomology
Journal of Parasitology
Journal of the American Mosquito Control
Association
Journal of the American Veterinary Medical
Association
Journal of Pharmaceutical Sciences
Laboratory Animal Science
Lancet
Medical Veterinary Entomology
Nature Medicine
Parasitology
Proceedings of the National Academy of Sciences
Tropical Doctor
Vaccine
Vaccine Adjuvants: Preparation Methods and
Research Protocols
Virology

Medical Chemical Defense (M3)

Archives of Toxicology
Comparative Medicine
Contemporary Topics
Epilepsy Research
Inhalation Toxicology
Journal of Biochemical and Molecular Toxicology
Journal of Toxicology, Cutaneous and Ocular
Toxicology

Medical Biological Defense (M4)

American Journal of Tropical Medicine and Hygiene
Antimicrobial Agents and Chemotherapy

Archives of Pathology and Laboratory Medicine
Clinical Infectious Diseases
Infection and Immunity
Journal of Immunological Methods
Journal of Infectious Diseases
Journal of Medical Entomology
Journal of Public Health Management
Journal of Virology
Laboratory Investigation; a Journal of Technical
Methods and Pathology
Military Medicine
Molecular and Cellular Probes Protein Expression
Purification
Science (AAAS)
Toxicology
Toxicon: Official Journal of the International Society
on Toxinology
Trends in Microbiology Vaccine
Virology

Human Systems Technology (M5)

Bioelectromagnetics
Comparative Medicine
European Version of the Journal of Applied
Physiology
Health Physics
IEEE Transactions on Biomedical Engineering
Journal of Defense Science
Journal of Neuroscience
Journal of Thermal Biology
Neuroscience Letters
Nonlinear Optics
Radio Frequency Radiation Dosimetry
Student Military Medical Journal

Combat Casualty Care (M6)

American Journal of Sports Medicine
American Society of Hematology
Chest
Circulation
Immersed
Journal of Liposome Research
Journal of Applied Physiology
Journal of Immunology
Journal of Infectious Diseases
Journal of Neuroimmunology

Journal of Pharmacology and Experimental
Therapeutics
Journal of Surgical Research
Journal of Trauma
Journal of Urology
Military Medicine
Nature Medicine
Neuroscience Letter
Perfusion
Society for Neuroscience Abstracts
Stroke
Transfusion
Undersea Hyperbaric Medicine

Ionizing Radiation (M7)

Advanced Space Research
Annals of the New York Academy of Sciences
International Journal of Immunopharmacology
Journal of Radiation Research
Military Medicine
Neurotoxicology
Radiation Oncology Investigations
Radiation Research

Medical Studies - Other (M8)

Ecotoxicology and Environmental Safety
Inhalation Toxicology
International Journal of Toxicology
Veterinary Pathology

Non-Medical Research - Physical Protection and Detection (N1, N2)

Health Physics
Journal of Biomedical Optics
Journal of Laser Applications
Shock

Non-Medical Research - Other RDT&E (N4)

Bulletin of Environmental Contamination and
Toxicology
Toxicological Sciences
Bioacoustics
Journal of the Acoustic Society of America
Marine Mammal Science
U.S. Navy Journal of Underwater Acoustics
Toxicologist
Journal of Applied Toxicology Science

PROCEEDINGS, ABSTRACTS, AND PRESENTATIONS

Clinical Medicine (C1)

American Society for Laser Medicine and Surgery
10th Annual Meeting
Association for Research in Otolaryngology
Circulation
Federation of American Societies for Experimental
Biology Journal
Fifth International Conference on Neuroprotection
Gastroenterology
Journal of Endodontics
Journal of Periodontology
Pediatric Research
Annual Meeting of the Society for Research on
Nicotine and Tobacco
Prosper Meniere Society
The Association for Research in Vision and
Ophthalmology Annual Meeting
Washington Psychiatric Society News

Clinical Surgery (C2)

47th Annual Kimbrough Urological Seminar
Federation of American Societies for Experimental
Biology Journal
Laboratory Animal Science
137th Annual Convention of the American Veterinary
Medical Association

Infectious Disease (M2)

100th Annual Meeting American Society for
Microbiology
100th General Meeting of the American Society for
Microbiology
16th Annual Symposium on Non-human Primate
Models of AIDS
1st International Congress on Infectious and Tropical
Diseases
35th Joint Conference of the U.S. - Japan Cholera and
Other Bacterial Enteric Infections Joint Panel
49th Annual Meeting of the American Society of
Tropical Medicine and Hygiene
49th Annual Meeting of the American Society of
Tropical Medicine and Hygiene
7th Conference on Retroviruses and Opportunistic
Infections
Abstracts of the 13th International Congress of the
International Organization of Mycoplasma
American Society of Gene Therapy
American Society of Microbiology General Meeting
American Society of Tropical Medicine and Hygiene,
48th Annual Meeting

Basic Aspects of Vaccines, 6th National Symposium
Federation of American Societies for Experimental
Biology Journal
International Conference on Emerging Infectious
Diseases (ICEID 2000)
Millennium Second World Congress on Vaccines
and Immunizations, Infectious Control World
Organization
National Institute of Allergy and Infectious
Diseases, National Institutes of Health
Sixth National Symposium: Basic Aspects of
Vaccines
Society for Neuroscience Abstracts

Medical Biological Defense (M4)

Sixth National Symposium: Basic Aspects of
Vaccines
Society for Neuroscience Abstracts
U.S. Army Medical Defense Bioscience Review

Human Systems Technology (M5)

21st Annual Meeting of the Bioelectromagnetics
Society
Abstracts of the 22nd Annual Meeting of the
Bioelectromagnetics Society
Bioelectromagnetics Society Twenty-Second Annual
Meeting
Federation of American Societies for Experimental
Biology (FASEB) Journal
Society for Neuroscience Thirtieth Annual Meeting

Combat Casualty Care (M6)

Advanced Technology Applications for Combat
Casualty Care Conference
Advances Ion Channel Research
Annual Meeting of the Society for Experimental
Biology
Disabled Submarine (DISSUB) Team Bi-Annual
Meetings
DoD Bioscience 2000 Medical Defense Review
DoD Surgeon Generals Conference
Experimental Biology 2000 Meeting
Federation of American Societies for Experimental
Biology (FASEB) Journal
Immunology 2000
NATO Conference on Hypo- and Hyperbaric
Research
Society for Neuroscience
Society for Neuroscience Abstracts
Society of Academic Emergency Medicine
Undersea Hyperbaric Medicine

Ionizing Radiation (M7)

International Conference on Low-level Radiation and Predictive Assays
Society of Experimental Biology and Medicine
Uniformed Services University of the Health Sciences
Research Day

Medical Studies - Other (M8)

Neuroscience Abstracts
Chemical Propulsion Information Agency
Toxicological Sciences

Non-Medical Research - Physical Protection and Detection (N1, N2)

Society for Risk Analysis Annual Meeting

Non-Medical Research - Other RDT&E (N4)

Toxicology and Risk Assessment Approaches for the 21st Century

2000 Conference on Topics in Toxicology Risk Assessment: Approaches for the 21st Century
Bottlenose Dolphin Reproduction Workshop
2000 Congress on Evolutionary Computation
Thirteenth Biennial Meeting of the Society for Marine Mammology
39th Annual Society of Toxicology Meeting
Conference on Toxicology and Risk Assessment
International Aviation Fire Protection Association, 1st Annual Meeting
National Conference on Aquaria, Fish Models of Human Disease
Pollution Prevention and Hazardous Waste Management Conference

Training/Instructional (T)

137th Annual Convention of the American Veterinary Medical Association

APPENDIX H

CHANGE TO DoD STANDARD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STANDARD PROTOCOL FORMAT



**OFFICE OF THE DIRECTOR OF
DEFENSE RESEARCH AND ENGINEERING**
3040 DEFENSE PENTAGON
WASHINGTON, D.C. 20301-3040

MAR 24 2000

Mr. Carlos J. Chapa
Technical Director, Audit Follow-Up and GAO Affairs
DoD Inspector General
400 Army Navy Drive
Arlington, VA 22202-2885

Dear Mr. Chapa:

Enclosed is a copy of the letter that changes the Department of Defense (DoD) Standard Institutional Animal Care and Use Protocol Format Instructions. The purpose of these changes is to implement the recommendations of the GAO report on the care and use of laboratory animals in DoD sponsored programs.

If you have any questions regarding our action on the GAO recommendations, please contact my regulatory affairs officer, CDR Doug Forcino, at 703-601-1724 or 703-588-7420.

Sincerely,

A handwritten signature in cursive script that reads "Delores M. Etter".

Delores M. Etter
Deputy Director
Defense Research & Engineering

Enclosure





**OFFICE OF THE DIRECTOR OF
DEFENSE RESEARCH AND ENGINEERING**
3040 DEFENSE PENTAGON
WASHINGTON, D.C. 20301-3040

MAR 24 2000

MEMORANDUM FOR SECRETARY OF THE ARMY
SECRETARY OF THE NAVY
SECRETARY OF THE AIR FORCE
DIRECTOR, ARMED FORCES RADIOBIOLOGY
RESEARCH INSTITUTE
DIRECTOR, DEFENSE ADVANCED RESEARCH
PROJECTS AGENCY
DIRECTOR, DEFENSE THREAT REDUCTION AGENCY
PRESIDENT, UNIFORMED SERVICES UNIVERSITY OF
HEALTH SCIENCES

SUBJECT: Change to DoD Standard Institutional Animal Care and Use Committee
(IACUC) Protocol Format Instructions

The following changes and additions to the standard protocol format instructions for the use of animals in research sponsored by the Department are hereby promulgated. These changes and additions are effective immediately.

Paragraph II.B. Literature Search

Delete: "A search of Federal Research in Progress (FEDRIP) and DTIC databases or their equivalent is required for DoD funded research. An additional search of the scientific literature (MEDLINE, GRATEFUL MED, MEDLARS, AWIC, etc) is highly recommended."

Replace with: "A search of the Biomedical Research Database (BRD) is required. In addition, a search of EITHER the Federal Research in Progress (FEDRIP) OR the Computer Retrieval of Information of Scientific Projects (CRISP) databases is required. Requirements for additional searches are at the discretion of the IACUC."

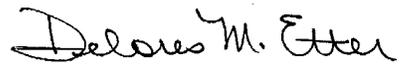
Paragraph V.B.5.a. Refinement

Amend this section to read:

- 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, also list the refinement alternatives that were considered but not adopted, and explain why they were not adopted.



Questions regarding this guidance should be directed to CDR Douglas Forcino,
(703) 588-7420, Regulatory Affairs Staff Officer.

A handwritten signature in black ink that reads "Delores M. Etter". The signature is written in a cursive style with a large initial 'D'.

Delores M. Etter
Deputy Director
Defense Research & Engineering

APPENDIX I

DoD INSPECTOR GENERAL RECOMMENDATIONS ON THE USE OF ANIMALS IN DoD MEDICAL RESEARCH FACILITIES AND CONTRACT RESEARCH FACILITIES

DoD Inspector General Recommendations on the Use of Animals in DoD Medical Research Facilities and Contract Research Facilities

MEDICAL RESEARCH FACILITIES

Recommendation 1: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should issue Department of Defense policy that requires every Department of Defense research facility to:

1. Support, and as necessary develop, animal care and use training programs, and encourage certification for all personnel involved in the care, use, and treatment of the animals; and
2. Develop a formal checklist to be used by the Institutional Animal Care and Use Committee when conducting its semiannual inspection. The published reports should document use of the checklist. All members of the Institutional Animal Care and Use Committee should sign the report that also includes a statement indicating there are or are not minority opinions.

Recommendation 2: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs) and the General Counsel, Department of Defense, should provide clear Department of Defense guidance concerning the requirements and qualifications of the non-affiliated member of the Institutional Animal Care and Use Committee. The guidance should establish eligibility requirements, professional qualification, and characteristics for committee members, and set the minimum number of non-affiliated members desired.

Recommendation 3: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should direct the Armed Services Biomedical Research Evaluation and Management Committee to develop a standardized, comprehensive Department of Defense research protocol request form and require its use by all Department of Defense research facilities.

Recommendation 4: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should ensure each research facility commander is provided with information concerning the commendable practices identified by the inspection teams for consideration in their animal care and use program.

CONTRACT RESEARCH FACILITIES

Recommendation 1: The Director of Defense Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should issue Department of Defense policy that requires the Military Departments and the research facilities operated by the Office of the Secretary of Defense to complete the following tasks before awarding any contract or grant that involves research using any live animals:

1. All extramural research proposals using live animals should be reviewed by a veterinarian trained and knowledgeable about laboratory animal medicine to ensure compliance with all Federal laws, and Department of Defense regulations and guidelines concerning the care and use of animals.

2. To ensure the facility is complying with the requirements in the Animal Welfare Regulation, the Department of Defense funding agency should contact the United States Department of Agriculture to obtain copies of the most recent inspection reports for a facility under consideration for a contract or grant.

Recommendation 2: The Director of Defense Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should issue Department of Defense policy that requires the Military Departments and research facilities operated by the Office of the Secretary of Defense to perform the following tasks after a contract or grant that involves live animal use is awarded:

1. A veterinarian knowledgeable about laboratory animal medicine should conduct site visits to evaluate the animal care and use program at contract research facilities using non-human primates, marine mammals, dogs, or cats; conducting research deemed sensitive; or cited by the United States Department of Agriculture as a research facility under investigation. The policy should include the requirements for the initial site visit and the conditions for follow-on site visits.
2. To ensure continued compliance with the Animal Welfare Regulation, the Department of Defense funding agency should contact the United States Department of Agriculture on a routine basis to obtain a copy of the most recent annual inspection report for each facility with an active contract.

Recommendation 3: The Director of Defense Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should direct the Military Departments and the research facilities operated by the Office of the Secretary of Defense to require that all contractor proposals for research using live animals include all the information contained in the standardized Department of Defense protocol request format.

APPENDIX J

**OCCUPATIONS OF IACUC MEMBERS
(VOTING AND NONVOTING)**

OCCUPATIONS OF IACUC MEMBERS (VOTING AND NONVOTING)

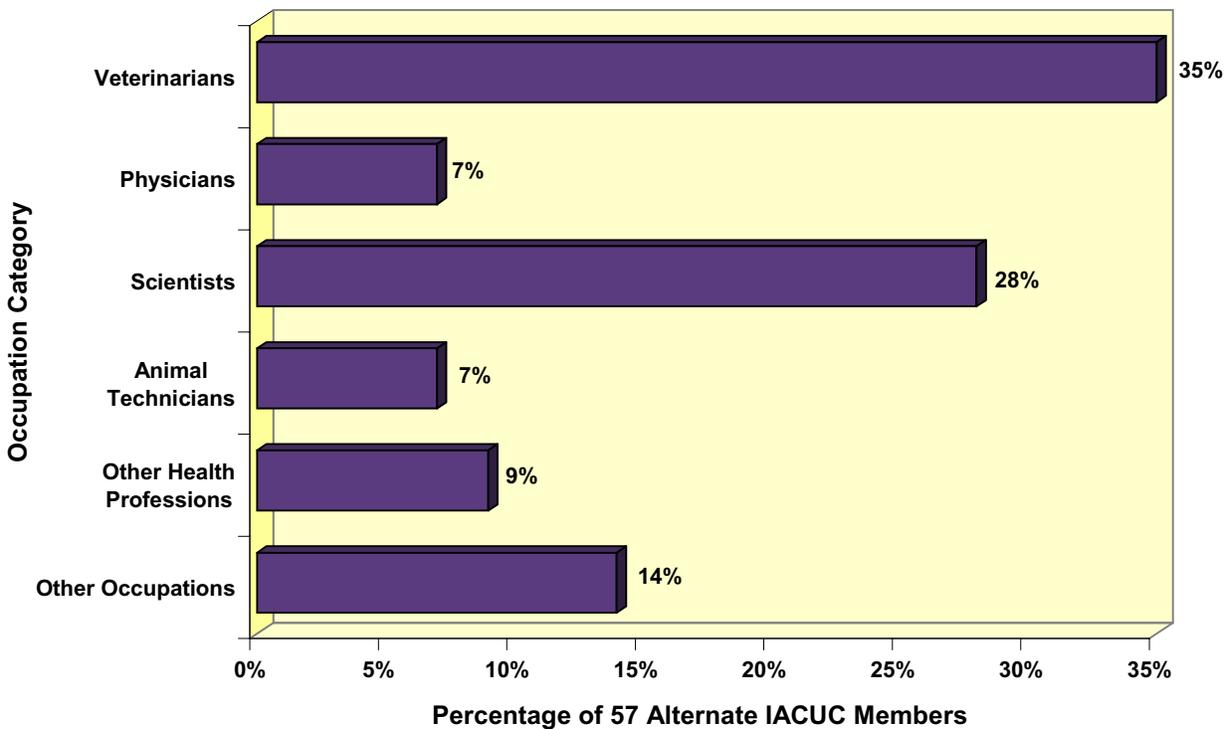
REGULAR IACUC MEMBERSHIP

As noted in Section III.2.2, veterinarians are most heavily represented among regular Institutional Animal Use and Care Committee (IACUC) members with full voting responsibilities. They can be expected to represent animal interests from the perspective of those intimately involved in housing, handling, and day-to-day care. Among physicians and scientists members, surgeons, microbiologists and physiologists predominate. These are important areas of expertise relative to research and animal care. Statisticians provide information on minimizing animal use while attaining research goals. The non-scientist's perspective is presented by those in occupations such as: administrator, secretary, teacher, attorney, salesman, wildlife technician, engineer, pharmacist, nurse, dentist, and clergy.

ALTERNATE IACUC MEMBERSHIP

The DoD requires all IACUC panels to have alternate members. These alternate members often serve to provide backup expertise for the IACUC. Many are veterinarians, medical personnel, or researchers. At some institutions, alternate members are used to train new members toward assuming regular voting IACUC positions. While they vote only if regular members cannot attend an IACUC meeting, alternate members often attend in a nonvoting capacity, providing additional expertise and experience to the panel and its deliberations. There were 57 alternate members in FY00. The occupations of alternate members under "Other Occupations," below, include: health care administrator, attorney, logistician, research administrator (non-scientific), secretary, and teacher.

OCCUPATIONS OF ALTERNATE MEMBERS



APPENDIX K

**DISSEMINATION OF INFORMATION ON
ANIMAL CARE AND USE**

DISSEMINATION OF INFORMATION ON ANIMAL CARE AND USE

All DoD institutions engage in training activities as required under DoD Directive 3216.1. Nonaffiliated members must receive at least 8 hours of training although both regular and alternate Institutional Animal Care and Use Committee (IACUC) members across the DoD logged in an average of about 12 hours of training time in FY00. This Appendix lists examples of training activities that were used over the course of the reporting year.

I. EXAMPLES OF TRAINING AND INFORMATION PROVIDED TO IACUC MEMBERS IN FY00

In FY00, DoD training activities reflected an emergence of computer-based resources. These can be expected to increasingly supplant traditional audiovisual resources such as slide presentations or videocassettes. Several types of computer-based tools are emerging.

- Computer-based (CD-ROM or Internet) training modules and publications are becoming available to offer information from IACUC fundamentals to the conduct of species-related activities. Generally, these resources are directed at non-veterinarians who wish or need to become more familiar with animal oversight, care, and use.
- List serves and forums have also become available to disseminate new information on developments in animal care and use, and to help address and resolve both specific and general concerns. Organizations providing listserv forums include the Applied Research Ethics National Association (ARENA), the American Association for Laboratory Animal Science (AALAS), the Scientists Center for Animal Welfare (SCAW), and the Lab Animal Welfare Training Exchange (LAWTE).
- The Internet provides ready access to important and useful documents for downloading. Regulatory documents are readily available online, and both government and non-profit organizations concerned with animal research maintain web-accessible policy and position documents that address a wide range of animal care and use concerns.

Traditional audiovisual materials still predominate. The American College of Laboratory Animal Medicine (ACLAM), the American Veterinary Medical Association (AVMA), and the Laboratory Animal Training Association (LATA) provide a variety of videocassette training modules. Institute-specific training modules, such as those at the U.S. Army Medical Research Institute for Infectious Diseases, have also been developed to address the unique facets of their respective animal research facilities and programs.

Finally, although IACUC members do not necessarily engage in animal handling, hands-on training resources are provided annually at the Walter Reed Army Institute of Research for both investigators and animal technicians (Appendix M). Both of these groups are represented on IACUC panels, bringing their unique perspectives to the animal use oversight process.

II. TOPICS COVERED IN DoD TRAINING

- 9 CFR
- Alternatives to animal use
- Alternatives to death as an endpoint
- Anesthesia, analgesics, and tranquilizers
- Animal care and handling
- Aseptic surgical techniques
- Asthma in animal caretakers
- AWA Regulations
- Biosafety
- Bloodborne pathogens,
- Colony access
- Definitions of reporting pain and distress
- DoD Directive 3216.1
- DoD Field Manual for Animal Care and Use
- Environmental enrichment
- Environmental/occupational health
- Ethics and animal welfare
- Euthanasia
- Facilities inspection
- Hazardous waste management
- Handling and restraint
- Housing of laboratory animals
- IACUC function

- IACUC issues in field biology
- Information resources
- Ionizing radiation
- Legal, regulatory and policy issues
- Literature searching
- Minimizing animal use
- Minimizing distress
- Pain and distress alleviation
- Principal investigator responsibilities
- Protocol development and review
- Public relations
- Radioisotope use
- Reporting suspected animal abuse or deficiencies
- Research resources
- Roles of different IACUC members
- Security
- Surgical techniques
- Statistical analyses
- Using other facilities for joint ventures to provide appropriate housing
- Vaccine development
- Veterinary care
- Whistle blower protection
- Zoonoses prevention and control

III. TRAINING ACTIVITIES AND RESOURCES REPORTED IN FY00

Interactive Teaching Strategies:

- One-on-one instruction by facility veterinarians
- Monthly mandatory IACUC meetings
- Sending committee members to outside training programs
- Supporting the attendance of both affiliated and nonaffiliated members at conferences
- Regular discussion of relevant animal use topics and issues at IACUC meetings
- Monthly discussions of journal articles and developments in animal use
- Tours of institutional animal facilities
- Attendance at veterinarian-developed courses
- Mandatory participation in list serves for all members
- Sharing of conference trip reports

Training Resources:

- Provision of hard-copy guides and books to each IACUC member
- Development of a mandatory CD-ROM computer-based interactive training course for animal care personnel and IACUC members
- Provision of journal subscriptions

Facilitation of Compliance, Oversight, and Forwarding of Complaints:

- Posters throughout the facility advising employees and the public on procedures for filing animal care and use complaints emphasize that individuals do not have to use the chain of command but can go directly to the IACUC chair or the Inspector General (IG)
- Posters announcing availability of anonymous “hot line” for registering concerns or complaints
- Annual briefings to all facility personnel on the IG complaint process
- Notices posted on bulletin boards throughout the facility on how to register a complaint
- Reading, review, and discussion of animal welfare regulations and policies

Investigator Training Activities:

- Mandatory investigator training courses
- Researchers and technicians required to have documented appropriate training before performing procedures on animals
- Requirement for research staff and graduate students to complete training courses on the humane and ethical use of animals prior to engaging in research activities
- Provision of operating instructions and manuals to each investigator
- Provision of library resources, including books, manuals, and videotapes
- Provision of regulatory and policy documents
- Provision of journal and newsletter subscriptions
- Provision of investigators’ procedural handbooks
- Briefings and veterinarian-directed discussions at IACUC meetings
- Provision of orientation training for new IACUC members
- Mandatory monthly seminars

Videotapes and Computer-based Modules:

- *The Humane Care and Use of Laboratory Animals* (Laboratory Animal Testing Association (LATA), 1991)
- *Animal Care Matters* (sic)
- American Veterinary Medical Association educational videos
- Caprine Zoonotic Diseases
- IACUC Composition and Regulations
- Essentials of Animal Use and Risks
- Laboratory Animals, Laws, Regulations, and Guidelines

Books and Publications:

- Silverman, J. *The IACUC Handbook*. CRC Press, 2000
- Podolsky, L. *The Care and Feeding of an IACUC*. CRC Press, 1999
- *Guide for Care and Use of Laboratory Animals* (National Academy Press, 1996)
- *New Investigator Training Handbook*
- *The Institutional Animal Care and Use Committee Guidebook* of the NIH Office of Laboratory Animal Welfare (OLAW; formerly part of the Office for Protection from Research Risks (OPRR)) and ARENA. NIH Publication No. 92-3415

Members Attended Meetings from One or More of the Following Organizations/Conferences:

- Acoustical Society of America
- American Association of Zoo Veterinarians
- American Association of Zoo Veterinarians
- Animal Welfare Information Center (Oct, 1999)
- Applied Research Ethics National Association (ARENA, March 2000)
- Association for Aquatic Animal Medicine

- European Aquatic Animal Medicine Association Laboratory Animal Medicine: Advancing Science and Animal Welfare in the 21st Century (May 2000)
- IACUC Issues in Field Biology, Medical University of South Carolina (Charleston, Sept 2000)
- International Marine Trainers Association
- Marine Mammal Society
- National Association for Biomedical Research (NABR) 2000 Conference (April 2000)
- National Capital Area Branch (NCAB) of the AALAS (Sept 2000)
- Ohio Forum on Animal Research Issues
- Public Responsibility in Medicine and Research (PRM&R, March 2000)

Workshops/Courses Cited:

- USUHS Protocol Writing Workshop
- Public Responsibility in Medicine and Research - IACUC 101 (ARENA/PRIM&R, March 2000)
- Various ARENA/PRIM&R IACUC 101 courses around the nation
- “Meeting the Information requirements of the Animal Welfare Act” (AWIC)
- Animal care and use courses sponsored by the NIH, AAALAC International, SCAW, and the USDA
- USAMRIID Investigator Training
- Animal Welfare and GLP Regulations (University of Pennsylvania)

Subscription to journals including one or more of the following:

- *Comparative Medicine*
- *COMP MED* (electronic newsletter)
- *Contemporary Topics*
- *Journal of the Acoustical Society of America*
- *Journal of the American Veterinary Medical Association*
- *Journal of Wildlife Diseases*
- *Journal of Zoo and Wildlife Medicine*
- *Lab Animal*
- *Marine Mammal Science*
- *NABR* (electronic newsletter)
- *PRIM&R* (electronic newsletter)
- *Soundings*
- *Veterinary Technician*

IV. ORGANIZATIONS PROVIDING COURSES AND/OR EDUCATIONAL TRAINING IN FY00

- Scientists Center for Animal Welfare (SCAW)
- American Association for Laboratory Animal Science (AALAS)
- USDA Animal Welfare Information Center (AWIC)
- Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)
- Public Responsibility in Medicine and Research (PRM&R)
- Applied Research Ethics National Association (ARENA)
- National Association for Biomedical Research (NABR)
- Laboratory Animal Welfare Training Exchange (LAWTE)

APPENDIX L

STATUS OF AAALAC ACCREDITATION OF DoD ANIMAL CARE AND USE FACILITIES

STATUS OF AAALAC ACCREDITATION OF DoD ANIMAL CARE AND USE FACILITIES

I. U.S. DoD Facilities Accredited by AAALAC

I.1 OSD Components:

- Armed Forces Institute of Pathology, Washington, D.C.
- Armed Forces Radiobiology Research Institute, Bethesda, MD
- Uniformed Services University of the Health Sciences, Bethesda, MD

I.2 U.S. Army:

- U.S. Army Research Institute of Environmental Medicine, Natick, MA
- U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD
- U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD
- U.S. Army Center for Environmental Health Research, Fort Detrick, MD
- U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD
- William Beaumont Army Medical Center, El Paso, TX
- Tripler Army Medical Center, Tripler Army Medical Command, Honolulu, HI
- Madigan Army Medical Center, Tacoma, WA
- U. S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD
- U.S. Army John F. Kennedy Special Warfare Center , Fort Bragg, Fayetteville, NC
- Walter Reed Army Institute of Research, Forest Glen, MD
- Brooke Army Medical Center, Fort Sam Houston, TX
- U.S. Army Medical Department Center and School, Fort Sam Houston, TX
- Dwight David Eisenhower Medical Center, Fort Gordon, GA
- U.S. Army Dugway Proving Ground, Dugway, UT
- U.S. Army Institute of Surgical Research, Fort Sam Houston, TX

I.3 U.S. Navy:

- Naval Dental Research Institute, Naval Training Center, Great Lakes, IL
- Naval Medical Center, Clinical Investigation Program, San Diego, CA
- Naval Medical Center, Clinical Investigation and Research, Portsmouth, VA
- Naval Medical Research Center, Fort Glen, MD
- Space and Naval Warfare Systems Center, San Diego, CA

I.4 U.S. Air Force:

- Air Force Research Laboratory, Wright-Patterson AFB, OH
- Air Force Research Laboratory, Brooks Air Force Base, TX
- Keesler Medical Center, 81st Medical Group, Keesler AFB, MS
- Wilford Hall Medical Center, 59th Medical Wing, Lackland AFB, TX
- David Grant Medical Center, 60th Medical Group, Travis AFB, CA
- U.S. Air Force Academy, Colorado Springs, CO

II. Overseas Facilities Accredited by AAALAC:

- Naval Medical Research Center Detachment, Lima, Peru
- Naval Medical Research Unit #2, Jakarta, Indonesia
- Naval Medical Research Unit #3, Cairo, Egypt
- Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

APPENDIX M

**THE 2000 WRAIR DoD LABORATORY ANIMAL CARE
AND HANDLING WORKSHOP SCHEDULE**

THE 2000 WRAIR DoD LABORATORY ANIMAL CARE AND HANDLING WORKSHOP SCHEDULE

LABORATORY ANIMAL TRAINING - THE CORE COURSE

In 1998, the WRAIR's Laboratory Animal Care and Use Committee updated and revised their policy on research personnel training. One of the outcomes of this revision was the creation of the WRAIR/NMRC's Animal Care and Use Program Web Page (<http://wrair-www.army.mil>; choose Animal Care and Use Program). The other new provision was the requirement that all personnel using or caring for animals in research take the Laboratory Animal Training Core Course every 3 years. To assist personnel in meeting this requirement, the "Core" course will be offered in five different formats:

- The Animal Care and Use Program (ACUP) Orientation Course
- Three "self-paced" methods - a study of the investigators' handbook, a study of the Web Page, and the "Issues Class on Line" available through the website. As this brochure went to press, the "Issues Class on Line" was not yet available. It is the Division's goal to make it so by January 2001.

THE ANIMAL CARE AND USE PROGRAM (ACUP) ORIENTATION

This course is offered monthly in conjunction with the Institute's required safety courses. The 2-hour course acquaints participants with the basic components of the WRAIR-NMRC Animal Care and Use Program. Speakers will briefly review the background for the Institute's ACUP policies and practices, cover critical aspects of animal use protocol development, explain the responsibilities of the WRAIR-NMRC Institutional Animal Care and Use Committee. Identify key personnel associated with the ACUP; provide a virtual tour of the Building 503 animal facilities, detailing standard practices for working in the facilities; and address a number of other topics critical to the ACUP. The course is open to investigators, technicians, and administrative personnel. There is no limit to class size. Location: Building 503 Auditorium.

Schedule: Odd Months

May 10
July 5
September 6
November 1

Even Months

June 7
August 2
October 4
December 6

Time:

0830-1030 HAZCOM
1100-1200 Fire Prevention
1300-1400 Bloodborne Pathogen
1430-1630 ACUP Orientation

0830-1030 ACUP Orientation
1130-1200 Bloodborne Pathogen
1300-1400 Fire Prevention
1430-1630 HAZCOM

SELF-GUIDED STUDY OF THE INVESTIGATORS' HANDBOOK

To obtain certification for the "Core Course," using the Investigators' Handbook format, please do the following:

Contact the Division of Veterinary Medicine's Education Resources Office at 301-319-9470/9760 to request the Investigators' Handbook and Quiz. The quiz will also be posted to the Laboratory Animal News Electronic Bulletin Board. The Handbook was last revised in 1997.

Complete the quiz and return it to Mrs. Terri Western, Division of Veterinary Medicine, WRAIR (fax 301-319-9980). The test will be graded and returned to you along with your certificate of completion. The test takes approximately 25 minutes to complete.

SELF-GUIDED STUDY OF THE ANIMAL CARE AND USE PROGRAM WEB SITE

To obtain certification for the “Core Course,” using the Animal Care and Use Program Web Site, please do the following:

Access the Animal Care and Use Program Web Page at <http://wrair-www.army.mil>, and click on the Animal Care and Use Program button. On the menu page, select Core Course Quiz. The quiz will be in Microsoft Word format and can be completed online, or printed and completed by hand.

The quiz should be returned to the Educational Resources Office, Division of Veterinary Medicine, WRAIR (fax 301-319-9980); email: Terri.Western@na.amedd.army.mil.

The test will be graded and returned to you along with your certificate of completion. The test takes approximately 25 minutes to complete.

NONHUMAN PRIMATES AND SAFETY BADGE CLASS

Special Health and Safety Notes - All participants attending the Primate and Safety Course **must** have had a negative TB test reading within the last 6 months prior to the date of the class.

The Primate Class is not recommended if you are pregnant (use of acyclovir if exposed to Herpes B), have recently been exposed to measles, or have the flu or other potentially contagious illness.

In addition to the standard clothing requirements, participants are advised not to wear dangling earrings or other jewelry when working with primates.

General Information - The Nonhuman Primate and Safety Badge Class includes a didactic, safety, and lab portion, focusing primarily on macaques. Individuals will take a short exam covering the special safety measures required for work with nonhuman primates. Upon passage of the exam, individuals will be issued a nonhuman primate room entrance authorization/medical alert badge. Further information about nonhuman primate safety issues or training in New World and other nonhuman primate species can be obtained by contacting the Division of Veterinary Medicine at (301) 319-9470. Class size limited to 10.

Time: 0830-1230

Schedule: 12 May 2000 - Bldg. 511 Classroom
14 July 2000 - Bldg. 511 Classroom
6 October 2000 - Bldg. 511 Classroom
1 December 2000 - Bldg. 511 Classroom
19 January 2001 - Bldg. 511 Classroom

RODENT CLASS (RATS, MICE, GUINEA PIGS)

Special Health and Safety Notes - A tetanus vaccination is not required for participation in the class; however, anyone working with rodents should have a current tetanus vaccination.

Allergies to rodents are fairly common. Protective clothing worn during the workshop will offer some protection. If you have known allergies to rodents or find that you are experiencing allergy symptoms, please notify the course instructor.

General Information - The Rodent Class is a general species-specific course that includes both a didactic and lab portion. Class size is limited to 10.

Time: 0830-1300

Schedule: 5 May 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
2 June 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
11 August 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
8 September 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
3 November 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
8 December 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
25 January 2001 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503

Note: A rodent workshop will be offered specifically for summer students. Location and date to be announced.

LAGOMORPH CLASS

Special Health and Safety Notes - Allergies to rabbits are fairly common. Protective clothing worn during the workshop will offer some protection. If you have known allergies to rabbits, or find that you are experiencing allergy symptoms, please notify the course instructor.

General Information - The Lagomorph Class is a general species-specific course that includes both a didactic and lab portion. Class size is limited to 10.

Time: 0830-1300

Schedule: 19 May 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
27 July 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
7 September 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
17 November 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
8 December 2000 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503
26 January 2001 - *Lecture:* Bldg. 511 Classroom *Lab:* Room GW50, Bldg. 503

Note: A lagomorph workshop will be offered specifically for summer students. Location and date to be announced.

ASEPTIC AND STERILE TECHNIQUES CLASS

General Information - The aseptic and sterile techniques workshop reviews the principles of aseptic and sterile techniques required by federal law to support rodent survival surgeries. Survival surgeries involving animals (other than rodents) must be performed in a dedicated surgery. The class includes both didactic and lab sections. Live animals are not used. The workshop is open to investigators and technicians. Class size is limited to 12.

Time: 0830-1230

Schedule: 25 June 2000 - Bldg. 511 Classroom
14 September 2000 - Bldg. 511 Classroom
26 October 2000 - Bldg. 511 Classroom
14 December 2000 - Bldg. 511 Classroom

SWINE CLASS

General Information - The Swine Class is a general species-specific course that includes both a didactic and lab portion. Class size is limited to 8.

Time: 0830-1330

Schedule: 21 May 2000 - Bldg. 511 Classroom
29 September 2000 - Bldg. 511 Classroom
9 November 2000 - Bldg. 511 Classroom

INTRODUCTION TO LABORATORY ANIMALS WORKSHOP

General Information - The Introduction to Laboratory Animals Workshop is offered for high school, college and other summer hire contract personnel. The course is general in scope and attendees do not need technician level skills to participant. In previous years, this course has been taken by students working in both animal and nonanimal use labs (students from the nonanimal labs took the course for general information and education).

The course provides students with a broad overview of laboratory animal care and use policies, practices, and procedures, and includes a tour of the animal facilities. The “hands-on” portion involves instruction in basic handling and care of rodents and rabbits. This course does not include instruction in research techniques and DoD workshop certificates are not given to attendees. Students actively involved in research must also take the regular DoD workshop for that species.

NOTE: Students who have any occasion to enter nonhuman primate rooms or work with nonhuman primates must have clearance from the Director, Division of Veterinary Medicine, and must take the regular DoD Nonhuman Primate Laboratory Animal Workshop.

Time: 0830-1330

Schedule: 28 June 2000 - Bldg. 511 Classroom
12 July 2000 - Bldg. 511 Classroom
13 July 2000 - Bldg. 511 Classroom

APPENDIX N

ANIMAL USE CATEGORIES

ANIMAL USE CATEGORIES

MEDICAL (M)

M1: Military Dentistry

Includes studies in the areas of:

- dental disease and management of dental emergencies
- testing medical devices for maxillofacial injury
- testing materials for maxillofacial injury
- surgical management of maxillofacial injury

M2: Infectious Diseases

Includes studies in the areas of:

- emerging infectious diseases of military importance
- vaccine development for prevention of bacterial sepsis and septic shock
- shigella vaccines
- malaria vaccines
- gonococcal peptide vaccine
- enterotoxigenic *E. coli* (ETEC) vaccine
- rickettsial diseases
- group A streptococcal vaccines
- polyvalent meningococcal vaccine
- prevention of *Campylobacter* diarrheal disease
- hepatitis virus vaccines
- establishment of diagnostic tests for infectious disease agents
- diagnosis of leishmaniasis
- development of drug therapies for infectious disease agents
- dengue virus vaccines
- viral hemorrhagic fever and encephalitis prevention and countermeasures
- identification and control of insect vectors of infectious diseases
- prevention of military HIV infection

M3: Medical Chemical Defense

Includes studies in the development of:

- medical countermeasures for vesicant agents
- medical pretreatment for cyanide
- prophylactic therapeutics for chemical agents
- reactive topical skin protectant
- medical countermeasures for respiratory agents
- chemical casualty management strategies and treatments

M4: Medical Biological Defense

Includes studies in the development of medical countermeasures for:

- *Yersinia pestis*
- brucellosis
- anthrax
- *Clostridium perfringens*
- Q-fever
- *Francisella tularensis*
- encephalomyelitis viruses
- variola
- *Filoviridae*
- physiologically active compounds
- sodium channel neurotoxins
- ricin
- staphylococcal enterotoxin B
- botulinum toxin
- venoms

M5: Human Systems Technology

Includes studies on:

- bioeffects of lasers
- laser impacts on performance
- treatment of laser-induced injury
- development of predictive models for a nonauditory exposure standard for blast overpressure
- development of occupational health protection criteria and exposure assessment technologies for toxic hazards arising from weapon systems and combat operations
- vibration
- bioeffects of electromagnetic radiation
- development of countermeasures for the effects of operational stress on military performance
- environmental injury
- development of methods, criteria, and predictive models for the risk of pulmonary injury in defeated armor scenarios

M6: Combat Casualty Care

Includes studies in:

- blood loss
- resuscitation
- secondary damage after hemorrhage
- soft tissue injury
- musculoskeletal injury
- combat stress injury
- burn injury
- anesthetics
- delivery systems

M7: Ionizing Radiation

Includes studies on:

- development of radioprotective compounds
- therapies for radiation-induced pathology
- bioeffects of ionizing radiation
- psychomotor effects of ionizing radiation
- mechanisms of radiation-induced pathophysiology

M8: Other Medical RDT&E

Includes studies in the areas of:

- breast cancer research
- neurofibromatosis research
- Gulf War illnesses
- laser research
- toxicology
- zoonosis
- free electron laser
- defense women's health research
- occupational medicine
- osteoporosis
- vectorborne diseases
- prostate cancer research
- ovarian cancer research
- environmental safety
- neurotoxin research
- bone health research
- disaster relief and emergency medical services
- Defense Health Research Program

NON-MEDICAL (N)

N1: Physical Protection

As previously indicated, excludes reporting military working animals and includes:

- developing hearing protection criteria
- mechanisms of and protection from military acoustic hazards
- ocular effects and performance of eye protective devices

N2: Physical Detection

Includes studies in the development of:

- biosensors
- chemical detection devices
- the Chemical Biological Mass Spectrometer (CBMS) detector
- auditory detection thresholds in marine mammals
- models of dolphin echolocation
- detection of biological warfare agents

N3: Offensive Weapons Testing

No studies were performed in this category in FY00.

N4: Other Non-Medical RDT&E

Includes studies in the areas of:

- telemedicine
- bioengineering
- controlled biological systems
- environmental assessment
- environmental research
- fish in marine and freshwater aquaria
- hearing testing
- improving learning and decision making
- jet fuel toxicity
- jet lag and wakefulness
- marine biology
- neural interface systems using rodent models
- neural science
- physical detection
- physiology
- minimally invasive therapy
- sonar
- toxicology
- underwater propulsion
- solvent toxicity
- propellant toxicity

CLINICAL INVESTIGATIONS (C):

C1: Clinical Medicine

Research conducted includes a wide variety of clinical medical diseases/conditions that are not necessarily unique to the military.

Includes studies in the areas of:

- burn treatment
- prophylaxis against toxic chemicals
- wound healing
- preservation of tissue sample morphology
- differentiation of brain tumors
- substances promoting repair of sound-sensing cells
- regulation of tracheal mucin secretion by retinoic acid
- breast cancer research
- mechanisms and treatment of renal pathophysiology
- effects of tumor necrosis factor on gonadotrophic activity
- treatment of immune-mediated hearing loss
- mechanisms of lung growth and compensation following injury
- testing of hepatitis-E vaccines

C2: Clinical Surgery

Includes studies in the areas of:

- adverse effects on wound healing of post-surgical treatments
- development of synthetic materials for surgical closures
- topical stimulants of skin healing following biopsies
- techniques of fiberoptic bronchoscopy
- laparoscopic cholecystectomy
- biomechanical and histological effects of artificial implants
- identification and development of improved implant materials
- evaluation of new techniques to remove seminal vesicle cysts
- electrohydraulic lithotripsy

C3: Other Clinical Investigations

- anesthetic response study
- microgravity induced musculoskeletal pathology

TRAINING AND INSTRUCTIONAL (T):

T1: Training, Education, and/or Instruction for Personnel

Types of training include:

- animal technician training
- training of special forces medics
- investigator training in proper techniques used with animals
- physician training in medical or surgical procedures, etc.

The training locations included DoD laboratories or medical centers. Does not include experimental or research-related work.

T2: Other Training/Instruction

No studies were performed in this category in FY00.

ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES (A):

A1: Adjuncts to Animal Use Research

Addresses those studies and uses that focus specifically on animal husbandry and care issues, and not directly on human medical, nonmedical, or training issues.

A2: Alternatives to Animal Investigation

Includes studies involving the use of animals that are designed to address directly and specifically issues of replacement, reduction, or refinement options for which animals are currently used; this classification does not include studies that are specifically directed at military RDT&E, clinical studies, or training requirements that may employ the animal alternatives of replacement, reduction, or refinement in the performance of the required protocols.

A3: Other Alternatives/Adjuncts

No studies were performed in this category in FY00.

CLASSIFIED SECRET OR ABOVE STUDIES (S):

S: Animals in Studies Classified SECRET or Above

Includes studies in which the information concerning the study may not be released for public knowledge because of the impact on national security.

ANIMAL BREEDING STOCK (B):

B: Animals Maintained for Breeding

Includes:

- animals maintained at the facility or supported through contract funds for breeding purposes to supply offspring to be used in animal-based research for particular work units or protocols
- breeding animals and offspring not assigned to specific work units or protocols

OTHER ANIMAL USE CATEGORIES (O):

O: Other Animal Use Purposes

Includes:

- animals awaiting assignment to protocols
- basic sleep research
- neurosciences
- quality assurance

APPENDIX O

SUMMARY OF ANIMAL USE DATA BY CATEGORY

SUMMARY OF ANIMAL USE DATA BY CATEGORY

A1	
Animals Reported	Animals Used
Chinchilla	83
Guinea Pig	9
Mouse	179
Rat	130
Total A1	401

A2	
Animals Reported	Animals Used
Fish	14,350
Frog	181
Rat	73
Total A2	14,604

B	
Animals Reported	Animals Used
Mouse	9
Rat	36
Total B	45

C1	
Animals Reported	Animals Used
Chinchilla	172
Dog	10
Ferret	25
Goat	1
Guinea Pig	94
Monkey	67
Mouse	5,935
Pig	138
Rabbit	27
Rat	4,511
Sheep	49
Vole	10
Total C1	11,039

C2	
Animals Reported	Animals Used
Chicken	251
Dog	36
Ferret	16
Goat	66
Guinea Pig	75
Monkey	78
Mouse	2,816
Pig	297
Rabbit	456
Rat	1,366
Total C2	5,457

C3	
Animals Reported	Animals Used
Rat	80
Total C3	80

M2	
Animals Reported	Animals Used
Bat	334
Bird	9
Chicken	370
Dog	45
Ferret	98
Goose	5
Guinea Pig	561
Hamster	878
Jird	3
Monkey	537
Mouse	67,663
Rabbit	121
Rat	1,293
Sheep	16
Total M2	71,933

M3	
Animals Reported	Animals Used
Fish	1,000
Guinea Pig	4,228
Monkey	60
Mouse	6,297
Pig	45
Rabbit	748
Rat	593
Total M3	12,971

M4	
Animals Reported	Animals Used
Chicken	48
Goat	7
Guinea Pig	1,950
Hamster	1,730
Monkey	490
Mouse	73,568
Pig	322
Rabbit	326
Rat	842
Sheep	30
Total M4	79,313

M5	
Animals Reported	Animals Used
Monkey	245
Mouse	342
Pig	47
Rabbit	105
Rat	1,645
Total M5	2,384

M6	
Animals Reported	Animals Used
Baboon	16
Cow	18
Dog	122
Goat	51
Guinea Pig	20
Mouse	1,525
Pig	984
Rabbit	83
Rat	7,312
Sheep	47
Total M6	10,178

M7	
Animals Reported	Animals Used
Dog	68
Guinea Pig	197
Mouse	13,519
Rat	351
Total M7	14,135

M8	
Animals Reported	Animals Used
Bird	434
Cat	100
Chicken	2,060
Chinchilla	10
Chipmunk	14
Cow	129
Dog	271
Frog	320
Goat	29
Guinea Pig	24
Hamster	3
Monkey	121
Mouse	101,773
Pig	149
Rabbit	270
Rat	15,397
Shrew	19
Squirrel	2
Vole	55
Total M8	121,180

N1	
Animals Reported	Animals Used
Deer	12
Monkey	49
Mouse	1,033
Pig	31
Rat	1,565
Total N1	2,690

N2	
Animals Reported	Animals Used
Dog	12
Mouse	140
Rat	530
Snake	23
Total N2	705

N4	
Animals Reported	Animals Used
Bat	14
Cow	8
Dog	20
Dolphin	33
Ferret	12
Fish	1,269
Frog	12
Guinea Pig	82
Monkey	46
Mouse	3,060
Pig	69
Rabbit	490
Rat	6,147
Sea Lion	10
Seal	2
Sheep	76
Vole	21
Whale	1
Total N4	11,372

O	
Animals Reported	Animals Used
Degu	12
Dog	5
Guinea Pig	8
Hamster	641
Monkey	139
Mouse	399
Pig	27
Rabbit	87
Rat	32
Snake	34
Total O	1,384

S	
Animals Reported	Animals Used
Goat	22
Monkey	2
Rat	42
Total S	66

T1	
Animals Reported	Animals Used
Bird	475
Cat	17
Chicken	21
Dog	72
Ferret	97
Frog	24
Gerbil	6
Goat	1,993
Goose	30
Guinea Pig	285
Hamster	32
Horse	2
Monkey	39
Mouse	761
Pig	719
Rabbit	605
Rat	632
Sheep	37
Vole	19
Total T1	5,866

Grand Total Animal Use/Research	365,803
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APPENDIX P

SPECIFIC ALTERNATIVES IMPLEMENTED IN FY00

SPECIFIC ALTERNATIVES IMPLEMENTED IN FY00

Replacement - The replacement alternative addresses supplanting animal use with nonliving systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale.

Alternative Subtype	Research Category	Alternatives
Biochemical/physical methods	C1	The use of molecular biological techniques to assess vasopressin receptor subtypes eliminates the need to do all characterization of hormone receptor subclassifications with pharmacological tools in the whole animal.
	M2	Antiviral drugs are initially tested in vitro for the ability to inhibit flavivirus replication. Drugs that do not show activity in vitro are not tested in animals.
Non-mammalian species or species lower on the phylogenetic scale	A2	The frog has replaced rats in study of the effects of toxicants on reproductive organs.
	M2	The use of the mouse as a model for ETEC, Campylobacter, and Shigella eliminates the need for large animals to be used in the initial vaccination and challenge trials, and allows for the initial screening of potential vaccine candidates against these bacteria before trials in large animals (e.g., monkeys) and man are conducted.
	M4	The use of BALB/c mice replaces monkeys in Ebola research experiments.
	M4	The use of mice for the study of antiviral drugs and viral pathogenesis replaces the use of non-human primates for these purposes.
	M4	The use of rabbits in anthrax vaccination studies reduces the number of nonhuman primates that would otherwise have been used in these efficacy studies.
	M6	The rat model replaced the pig model in decompression sickness research.
Replacement slaughter house animal parts	T1	Ophthalmology training is accomplished by making use of bovine eyes acquired from a local slaughter house.
Species replaces companion animals	T1	Use of the anesthetized ferret model in the pediatric intubation laboratory replaces the previous companion animal model, the cat.

Alternative Subtype	Research Category	Alternatives
Replacement using in vitro cell cultures	M4	Cell culture growth of antibody producing hybridomas in flasks replaces the requirement for mice to make ascites for the production of monoclonal antibodies.
	M4	Nonanimal systems such as cell culture for virus titrations and plaque reduction neutralization assays eliminate the use of animals for these determinations.
	M4	Cell culture assays for determining the neutralization titers of sera replace the requirement for animals.
	M4	Cell culture growth of antibody-producing hybridomas in hollow fiber units replaces the requirement for mice to make ascites for use in the passive transfer studies.
	M4	Cell culture studies are used to characterize the infectivity of VRP preparations before animals are used.
Substitution of another animal species or the use of humans	M2	Vaccines are screened and proven to be efficacious and safe in rodents prior to being tested in nonhuman primates.

Reduction - Decreasing the number of animals used through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

Alternative Subtype	Research Category	Alternatives
Changes in endpoint measurements	C2	In a study of the pharmacokinetics of Taxol-Coated Stents, early time points are used first and stents analyzed.
Timed matings	B	When breeding guinea pigs, males and females are paired only when there is a projected need for offspring for use in experiments, reducing the number of animals that might be needlessly culled.
Animal sharing	T1	Following the use of goats in the Emergency Surgical Procedures Laboratory, limbs are harvested and used in the Animal Care Specialist wound management training.
Substitution of computer simulation, models or other technologies	M4	Use of whole-body plethysmography to measure individual minute volume and breathing rate to determine accurately the inhaled dose for each nonhuman primate allows the minimum number of animals to be used to obtain statistically significant results.
	M6	The study of resuscitative therapies for combined traumatic brain injury and hemorrhagic hypotension uses laser-doppler flowmetry for the measurement of cerebral blood flow, which allows for continuous measurements without the removal of brain tissue.

Alternative Subtype	Research Category	Alternatives
	T1	In the Ocular Trauma course, artificial model eyes are used prior to using animals in lab. Laboratory also uses a skull model for orbital floor repair and floor implant replacement training instead of animals.
	T1	The use videotapes and didactic course material prior to using animals to teach techniques in animal care and use.
	T1	In dental readiness training, mannequins are used to give the students some experience in establishing airways and reduce the number of animals needed.
	T1	In microvascular and microneural anastomosis training, principal investigators use vessel models to reduce the number of rats required.
Utilization of alternative biological testing methods	C2	Anti-costimulatory receptor monoclonal antibody development employs <i>in vitro</i> cell cultures to reduce the number of animals used.
	M2	In developing drugs against militarily relevant diseases, novel compounds are screened by a variety of <i>in vitro</i> methodologies and only those that show potential are tested in live animals.
	M2	Membrane feeding of one colony two times per week reduces the total number of hamsters necessary to maintain the insect colony.
	M2	The use of the “limit test” allows for less mice to be used to determine the challenge dose in mice than when using the classical LD ₅₀ .
	M2	In a study on preparation of Hyperimmune Adenovirus rabbit antiserum, a single control host cell immune antiserum is used for all the adenovirus rabbit anti-adenovirus sera reducing the number of rabbits used.
	M4	In the production of murine hybridomas secreting human antibodies, alternative techniques such as the use of hollow fiber filters are used whenever feasible or useful.
	M6	Dive profiles are selected to produce a high incidence of Decompression Sickness (DCS) (>50%) in order to keep the sample size low.
	M7	Research on radiation prophylaxis and therapy uses <i>in vitro</i> cell cultures to reduce the number of animals used.

Refinement - Procedures or measures taken to eliminate or minimize pain or distress in the animal(s) or enhance animal well-being. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, or the use of adjusted early experimental endpoints.

Alternative Subtype	Research Category	Alternatives
Environmental enrichment	T1	A unique housing pyramid that was created using Vari-Kennels offers multiple horizontal surfaces and “caves” that are frequently utilized by our group of cats used in intubation training.
Increased training for animal technicians to increase skills	T1	Training for researchers and animal care personnel in humane techniques for handling nonhuman primates, rodents and lagomorphs.
Reduce distress	C1	The investigator conditions the utilized chinchillas to the novelty of the sound exposure cage and separation from conspecifics in order to reduce separation anxiety.
	C1	A noninvasive method (tail cuff) is used to measure blood pressure rather than an invasive method.
	C1	Blood and urine samples are obtained via indwelling catheters that eliminate the need for repeated invasive manipulations of the animal to collect these samples.
	M2	Hamsters are given a 3-week rest period for recovery from providing blood meal for insect colonies.
	M2	Body weights are taken on a scheduled basis to help assess health status, prevent an overgrowth of tumors, and determine an appropriate endpoint before the animal begins experiencing distress.
	M2	The use of membrane feeding for mosquito colonies results in animals not being exposed directly to mosquitoes.
	M3 & M4	The use of radiotelemetry allows enhanced data collection with a significant decrease in distress to the animal.
	M4	The use of implanted osmotic pumps for prophylactic administration of antiviral drugs in mice avoids repeated medication dosing and minimizes animal handling and distress.
	M6	In studies on photodynamic therapy, the initial work is done <i>in vitro</i> (bacterial cultures) to determine efficacy of the laser (light wavelength) and photosensitizer against the selected bacterial strain.
	M6	Visual discrimination data are collected continuously throughout light and dark cycles from rats housed in their “home” conditioning chambers. This approach to behavioral assessment maximizes data obtained from each subject while minimizing handling and distress.
	M7	Use of a drug depot device reduces stress from handling for multiple drug dosings.
N1	Noninvasively measuring internal body temperature through the use of diffusion magnetic resonance imaging to measure the temperature increase resulting from exposure to microwave fields.	

Alternative Subtype	Research Category	Alternatives
Reduce pain	M2	Neutralizing antibody titer is used as the principal indicator of an immune response to dengue vaccines.
	M4	Chicks are anesthetized prior to mosquito feeding.
	M4	Goats and rabbits receive analgesics to relieve more than momentary pain or distress associated with inoculation.
	M4	At the onset of clinical signs, as determined by the attending veterinarian, animals receive analgesia during the course of infection to alleviate pain.
	M6	The antibody secreting cells are immortalized through hybridoma technology and reduce the need to repeatedly immunize mice to produce the required antibody.
	N4	To establish baseline electrophysiological data in selected animal species surface and subdermal electrodes are used.
	T1	During tracheal intubation training, a minimum interval of 3 weeks between training sessions is required for each animal.