

**Department of Defense
Animal Care and Use Programs 1996**

**Report to the Senate Armed Services
Committee and the House of Representatives
National Security Committee**

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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.

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.

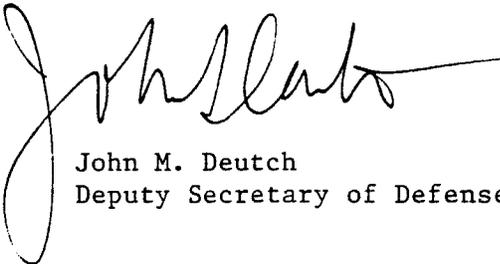
b. Designate the Commander, U.S. Army Veterinary Command/Director, DoD Veterinary Services Activity, a Field Operating Agency of the Army, Office of the Surgeon General who shall serve as a consultant to the Assistant Secretary of Defense for Health Affairs and the Director, Defense Research and Engineering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

Apr 17, 95
3216.1 (Encl 1)

(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

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

**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**



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.

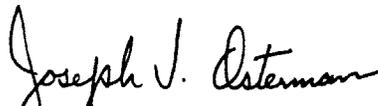
b) All DoD component facilities that utilize animals in research, testing and training shall implement the DoD standardized semi-annual program review checklist appended as attachment (3) immediately. Accompanying the checklist is a detailed outline of program review as contained in the NIH Guide for 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 -

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
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=====

GENERAL COMMENTS:

Appendix E

U.S. Government Principles for Animal Use

Appendix E

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 Animal Care and Use Programs

Appendix F

Benefits of DoD Animal Care and Use Programs

Medical

Military Dentistry

Development of an X-ray digital subtraction radiography for efficient detection and treatment of dental disease

Infectious Disease

Development of a vaccine for traveler's diarrhea and *E. coli*

Participation in Phase 2, 3, 4 drug evaluations

Identification of new agents for drug delivery systems

Identification of new species of bacteria

Establishment of colonies for laboratory models of malaria

Understanding the seasonal and geographic patterns of infectious diseases

Identification of Oropouche, dengue, Mayaro, Venezuelan, Equine Encephalitis and Yellow Fever

Maintain worldwide resources and reference laboratories

Providing ELISA kits and training to countries experiencing epidemics

Development of a pre-erythrocyte malaria vaccine in monkeys then in humans

Development of test kits for specific organisms allowing for rapid action/treatment

Understanding of the anthropod transmitted rickettsial disease

Understanding of the immune system antibody binding

Production of serum samples to characterize virus proteins as antigens

Understanding of the immune components and their ability to class switch

Chemical Defense

Establishment of potential hazards to humans by contamination and establish clean-up to manage by-products of military nerve agents

Identification of treatment compounds against lung injury by sulfur mustard gas

Biological Defense

Identification of medical countermeasures for botulinum toxin

Genetic engineering of vaccine candidates

Participation in Phase 2, 3, 4 drug evaluations

Human Systems Technology

Understanding of behavioral modification and identity of performance deficits

Identification of heart risks from arrhythmia's in pilots with irregular heart rhythms and validate aeromedical standards

Understanding of the circadian systems

Understanding of decompression and predict susceptibility to oxygen seizures

Understanding of thermal stress to reduce or control occurrence of cold induced peripheral vasoconstriction

Detect visual impairments caused by high G loads

Revision of the safety limit standards for nonionizing electromagnetic radiation in radio frequency and microwave range for communication, target acquisition, electronic warfare

Development of a model of conditioned defeat to diagnosis combat stress reactions

Assessment of Army weapon systems for health effects related to inhalation injury and blast overpressure research

Biomonitoring to protect receiving water
Provide information involving environmental effects of military activities
Identification of pharmacological agents to improve performance during rapid deployment
Identification of a model to verify laser systems with new characteristics and frequency
Understand the effects of environmental and operational stress

Combat Casualty Care

Identification use of artificial blood with liposome encapsulated hemoglobin
Prevention of organ damage after exposure to toxins, trauma, blood loss, ischemia and adverse environmental conditions
Performance of trauma surgery remotely using telepresence technology
Development of a model battlefield hemorrhagic shock
Development of a prototype Life Support for Trauma and Transport
Development of a process for producing blood substitute that can be freeze dried, is nontoxic, and universal
Identification of a mechanism for rapid cellular responses to trauma
Improvement of care of burn patients
Develop a better understanding of epidemiology of burn wound infection, postburn hypermetabolism and nutritional requirements
Understand acute hemorrhage management related to oxygen administration to maintain blood pressure
Understand the mechanics of trauma and thermal injuries with complications

Ionizing Radiation

Understanding of the bioeffects of ionizing radiation
Development of protective compounds

Other Medical Research

Investigate exposure of compounds causing Gulf War Syndrome
Understand the effects of maternal hormones at different stages of gestation
Identify relationship between mycoplasma infection and oncogenesis
Provide information to improve understanding of pulmonary hypertension in newborns
Development of an animal model for human mental retardation research
Development of an experimental model for seizures

Clinical Medicine

Develop and design coronary intervention devices and treatment of heart disease
Understand pathophysiology and management of respiratory injury
Determine the effects of anticoagulants and kidney disease related to incidence of severe intratracheal hemorrhage after fiberoptic transbone biopsy
Understand genetic hypertension to identify management techniques for high blood pressure
Identification of the mechanics underlying role of nitric oxide synthases in pulmonary hypertension
Test ventilator strategy with relevance to injured soldier
Development of therapeutic strategies to limit restenosis and vascular injury

Clinical Surgery

Prevent post-operative intra-abdominal adhesions
Develop techniques in balloon angioplasty catheter cannulation/decannulation complications
Determine the effects and toxicity of local anesthetics
Provide better understanding of general anesthetics during surgery, decreasing treatment time and prepare to perform mission in wartime environment

Non-Medical

Evaluate the effects of noise-induced stress
Determine the physical functionality of military smart sensors

Assess the toxicity risks of environmental compounds released
Determine the impact of munitions compounds and breakdown products on the environment
Replace Halon 1301 in fire extinguishing systems
Validate a model to identify noise hazards by military and civilian communities
Train, care and use marine mammals to provide economical means of underwater surveillance, object detection and marking

Training

Graduate medical training in surgical techniques, emergency surgery, obstetrical surgery dentistry, pediatrics, internal medicine
Advanced trauma life support training
Pediatric advanced life support training
Special Forces medical training
Education of military academy students in life sciences
Medical readiness training to support peacetime disasters and wartime contingencies
Education for front line lifesaving skills
Train medical professionals in thorascopic and percutaneous tracheotomy techniques in emergency situations
Fulfill training requirements subboard of Neonatal-Perinatal medicine
Developed cognitive architecture models of learners for use in embedded training systems

Alternatives

Develop alternatives to using higher phylogenetic animals
Develop single neuronal electrophysical recording technique in unanesthetized animals
Develop diaphragmatic electromyographic recording technique in freely behaving animals
Use tissue culture techniques to decrease use of higher phylogenetic animals
Produce, review and evaluate protocols to teach personnel safe and appropriate handling techniques for care, restraint, manipulation and sampling of animals

Appendix G

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

Appendix G

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 H

Nonaffiliated IACUC Members Professions

Appendix H

Nonaffiliated IACUC Members Professions

Accountant
Administrative Assistant
Aerobic Instructor
Attorney
Biologist
Biostatistician
Chaplain
Chemist
Communications Expert
Dentist
Editor
Engineer
Game Warden
Health Services Administrator
Homemaker
Hospital Corpsmen
Information Systems Specialist
Lab Scientist
Manpower Management Analyst
Medical Records Administrator
Medical Supply Company Owner
Microbiologist
Nurse
Personnel Consultant
Public Affairs Officer
Social Worker
Stable Manager
Supply Policy Chief
Teacher
Veterinarian

Appendix I

Dissemination of Information on Animal Care and Use

Appendix I

Dissemination of Information on Animal Care and Use

- 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 Institutional Animal Care and Use Committee (IACUC) chairman or the Inspector General (IG).
- 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
- Mandatory investigator training courses
- Mandatory monthly seminars
- Researchers and technicians required to have documented appropriate training before performing procedures on animals
- Research staff and graduate students required to attend a training course on the humane and ethical use of animals prior to engaging in research activities
- Provide each investigator with operating instructions and manuals
- Posters announcing availability of anonymous "hot line" for registering concerns/complaints
- Videotapes
- Investigators' handbooks
- Directed discussions at IACUC meetings
- Newsletters such as Scientists Center for Animal Welfare

Appendix J

The 1996 WRAIR DoD Laboratory Animal Workshop Schedule

Appendix J

The 1996 WRAIR DoD Laboratory Animal Workshop Schedule

<u>COURSE SUBJECT</u>	<u>DATES</u>
Nonhuman Primates & Safety Badge Class 0830-1230	27 March 1996 27 June 1996 11 July 1996 31 October 1996 16 January 1997
Rodents (Rats, Mice, Guinea Pigs) 0830-1300	4 April 1996 13 June 1996 18 July 1996 22 August 1996 18 October 1996 21 November 1996 24 January 1997
Lagomorphs 0830-1200	1 May 1996 28 June 1996 12 July 1996 27 September 1996 6 December 1996
Ovine 0800-1300	15 May 1996 4 October 1996
Swine 0800-1300	11 April 1996 6 June 1996 30 August 1996 22 November 1996
Issues in Laboratory Animal Care and Use 0830-1130	3 May 1996 19 July 1996 11 October 1996 17 January 1997
Writing an Animal Use Protocol Using the DoD Template 1200-1330	3 May 1996 19 July 1996 11 October 1996
Aseptic Techniques for Rodent Procedures 0830-1300	23 May 1996 17 July 1996 24 October 1996
Operating Room Sterile Techniques 0830-1230	8 May 1996 14 November 1996

Introduction to Laboratory Animals Workshop for Summer Students (Includes a "hands on" handling portion for rodents & NHP safety briefing/introduction) Class is designed for high school students and college students who have never worked in a laboratory environment before. Upper level college students, and students with previous experience may take this class if they wish, but should also take the regular workshops.

0830-1200
1230-1600

9 July 1996
9 July 1996

Appendix K

IACUC Training and Information

Appendix K

IACUC Training and Information

Non-affiliated IACUC Member Training Recommendations

The following are some example topics and resources which would fulfill the Congressionally mandated 8 hour training requirement for any new non-affiliated IACUC members. This is just one example of a program which would fulfill this training.

Topics:	Resources:
1. Humane Care and Ethics Issues Dealing with Animal Use (This block should be NLT 4 hours long)	<ul style="list-style-type: none">- Video (40 min) "IACUC Functions and the Humane Care and Use of Animals" available from the Laboratory Animal Training Association (LATA)- Questions and answers with the attending veterinarian- USAMRIID slide set (~200 slides covering Surgery, Euthanasia, Ethics, Pain and Distress)- Education and Training in the Care and Use of Laboratory Animals (Nat. Acad. Press, 1991)
2. Regulatory Responsibilities and Protocol Review Techniques (This block should be NLT 4 hours long)	<ul style="list-style-type: none">- Overview of DoD protocol format with the attending veterinarian- Lab animal protocol review articles (available from the editor as a bound notebook with 2 yrs of reviews)- USAMRIID slide set covering responsibilities, laws and regulations (~100 slides)
3. Facility Familiarization Tour	<ul style="list-style-type: none">- Attending veterinarian, facility manager, IACUC members
4. Basic Husbandry and Techniques of Laboratory Animals	<ul style="list-style-type: none">- LATA video tapes and script- ACLAM slide sets with audio cassettes- USAMRIID slide set
5. Documentation of Training	<ul style="list-style-type: none">- Each institute will develop a checklist and sign in logo to verify training received

Additionally, we recommend individual institute supplement in-house training programs by sending IACUC members to outside meetings such as PRIM&R/ARENA and AALAS.

Examples of Training and Information Provided to IACUC Members

- OPRR Institutional Animal Care and Use Guidebook
- NIH Publication 85-23, Guide for the Care and Use of Laboratory Animals
- PHS Policy on Humane Care and Use of Laboratory Animals
- Animal Welfare Act
- Local manuals on care and use of research animals
- The Journal "Lab Animal"
- Newsletter from the National Association for Biomedical Research
- Videotapes
- AAALAC program description
- One-on-one briefings
- Quarterly ethics workshop
- Ethics in research training courses
- Copy of DoD Regulation on use of animals in research
- Funded attendance at workshops by Scientists Center for Animal Welfare
- Funded attendance at the Public Responsibility in Medicine and Research conference "Animal Research Committees: Ethics, Education and Economics"
- Provided course "Animals in Medical Research - Guidelines" 3.5 hour course at National Naval Medical Center
- Provided continuing education training material to each member monthly
- Journal articles and newsletters provided to members and discussed at the committee
- Provided membership in the American Association of Laboratory Animal Science
- ILAR Publication - Education and Training in the Care and Use of Laboratory Animals, NRC and ILAR

Appendix L

Journals with DoD Animal Research Publications

Appendix L

Journals with DoD Animal Research Publications

Academic Emergency Medicine
American Industrial Hygiene Association Journal
American Journal of Physiology
American Journal of Respiratory Cell and Molecular Biology
American Journal of Tropical Medicine and Hygiene
American Sociological Review
Analytical Biochemistry
Anesthesiology
Annals of Tropical Medicine and Parasitology
Archives of Biochemistry and Biophysics
Archives of Oral Biology
Aviation, Space and Environmental Medicine
Biochemical Journal (London)
Biochemistry and Biophysical Research Communications
Biochemistry and Molecular Biology International
Bioelectromagnetics
Biomedical Chromatography
Biotechnology and Applied Biochemistry
Brain Research Bulletin
British Medical Journal
Carcinogenesis
Cerebral Cortex
Chemical and Biological Interactions
Chemical Senses
Chemosphere
Chest
Chirality
Clinical Chemistry
Clinical Infectious Diseases
Clinical Pharmacology and Therapeutics
Computer Methods and Programs in Biomedicine
Critical Care Medicine
Cytokine
Digestive Disease and Sciences
Drug and Chemical Toxicology
Drug Metabolism and Disposition
Electrophoresis
Environmental Health Perspectives
European Journal of Applied Physiology and Occupational Physiology
Experimental Hematology
Experimental Parasitology
FASEB Journal
Fertility and Sterility
Free Radical Biology and Medicine
Fundamental and Applied Toxicology
Human and Experimental Toxicology

Immunologic Research
Immunology
Immunology Today
Infection and Immunity
Infections in Urology
Inflammation
Inhalation Toxicology
International Journal of Radiation Biology
Investigative Ophthalmology and Visual Science
Journal of American Mosquito Control Association
Journal of Applied Physiology
Journal of Biological Chemistry
Journal of Clinical Investigations
Journal of Clinical Microbiology
Journal of Dental Research
Journal of Experimental Medicine
Journal of Immunology
Journal of Infectious Diseases
Journal of Investigative Dermatology
Journal of Leukocyte Biology
Journal of Medical Entomology
Journal of Medical Primatology
Journal of Membrane Biology
Journal of Neuroimmunology
Journal of Parasitology
Journal of Physiology
Journal of Radiologic
Journal of Rheumatology
Journal of Surgical Research
Journal of the American College of Cardiology
Journal of the American College of Toxicology
Journal of Toxicology and Environmental Health
Journal of Trauma
Journal of Urology
Journal of Vascular Surgery
Laboratory Animal Science
Lancet
Laryngoscope
Life Sciences
Mayo Clinic Proceedings
Military Medicine
Molecular Medicine
Neuropeptides
Neuroscience Letters
New England Journal of Medicine
Oncology
Parasitology Today
Pediatric Research
Pediatrics
Periodontology
Physiology and Behavior
Psychosomatic Medicine
Pulmonary Diseases and Disorders

Risk Analysis
Science
Shock
Structure
Techniques in Neurology
Tissue and Cell
Toxicologic Pathology
Toxicologist
Toxicology
Toxicology and Applied Pharmacology
Toxicology and Industrial Health
Toxicology Letters
Toxicology Methods
Toxicon
Undersea and Hyperbaric Medicine
Veterinary Pathology

Appendix M

Status of AAALAC Accreditation of DoD Facilities

Appendix M

Status of AAALAC Accreditation of DoD Animal Care and Use Facilities

I U.S. DoD Programs 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
- U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD
- U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, MD
- U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD
- William Beaumont Army Medical Center, Department of Clinical Investigation, Biological Research Service, El Paso, TX
- Tripler Army Medical Center, Tripler, Army Medical Command, Honolulu, HI
- Fitzsimons Army Medical Center, Aurora, CO
- Laboratory Animal and Surgery Service, Department of Clinical Investigations, Madigan Army Medical Center, Tacoma, WA
- U. S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD
- U.S. Army 1st Special Warfare Training Group, Fort Bragg, Fayetteville, NC
- Walter Reed Army Institute of Research, Washington, D.C.
- Department of Clinical Investigation, Brooke Army Medical Center, Ft. Sam Houston, TX
- U.S. Army AMEDD Center and School, Ft. Sam Houston, TX
- Dwight David Eisenhower Medical Center, Fort Gordon, GA

- U.S. Army Dugway Proving Ground, 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 Institute, Bethesda, MD
- Naval Command, Control and Ocean Surveillance Center, San Diego, CA

I.4 U.S. Air Force:

- Armstrong Laboratory - Wright-Patterson, Wright-Patterson AFB, OH
- Armstrong Laboratory - Brooks, Brooks Air Force Base, TX
- Clinical Research Laboratory, 81st Medical Group, Keesler AFB, MS
- Clinical Investigation Directorate, Wilford Hall Medical Center, Lackland AFB, TX
- Clinical Investigation Facility, 60th Air Mobility Command, Travis AFB, CA
- U.S. Air Force Academy, Colorado Springs, CO

II Overseas Programs Accredited by AAALAC:

- Naval Medical Research Institute Detachment, Lima, Peru
- Naval Medical Research Unit #2, Jakarta, Indonesia
- Naval Medical Research Unit #3, Cairo, Egypt

III Overseas DoD Program Not AAALAC Accredited:

- Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, has applied for AAALAC accreditation

Appendix N

Animal Use Categories

Appendix N

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
- a medical pretreatment for cyanide
- prophylactic therapeutics for chemical agents

- a 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 non-auditory exposure standard for blast over-pressure
- 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
- pathophysiology
- cognitive neuroscience
- Gulf War Syndrome
- laser research
- toxicology
- zoonosis
- free electron laser

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 performed in this category

N4: Other Non-Medical RDT&E

Includes studies in the areas of:

- environmental toxicology
- marine biology
- human systems technology
- acoustics signal processing
- chronobiology
- audiology
- pressure biology
- biological sensors
- computational neuroscience
- neurobiology
- spatial orientation
- sleep research
- biocatalysis

CLINICAL INVESTIGATIONS (C):

C1: Clinical Medicine

Research conducted includes a wide variety of clinical medical diseases/conditions which were 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

None in FY96

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

None in FY96

ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES (A):

A1: Adjuncts to Animal Use Research

Addresses those studies and uses which focused specifically on animal husbandry and care issues,

and not directly on human medical, non-medical, or training issues.

A2: Alternatives to Animal Investigation

Includes studies which involve the use of animals that are designed to address directly and specifically issues of reduction, refinement, or replacement 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 refinement, reduction, or replacement in the performance of the required protocols.

A3: Other Alternatives/Adjuncts

None in FY96

CLASSIFIED SECRET OR ABOVE STUDIES (S):

S: Animals on 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: Animal Maintained for Breeding

Includes:

- large 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
- environmental monitoring
- quality assurance

Appendix O

Summary of Animal Use Data by Category

Appendix O

Summary of Animal Use Data by Category

(M1) MILITARY DENTISTRY				Total:245
<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>	
MOUSE	206	RAT	30	
RABBIT	9			

(M2) INFECTIOUS DISEASES				Total:87,527
<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>	
BIRD	4	HAMSTER	1,926	
BURRO	2	HORSE	22	
CHICKEN	274	MOUSE	81,628	
COW	9	NON-HUMAN PRIMATE	873	
DOG	15	PIG/SWINE	138	
GERBIL	21	RABBIT	470	
GOAT	34	RAT	718	
GOOSE	23	SEA SLUG	22	
GUINEA PIG	1,268	SHEEP	80	

(M3) MEDICAL CHEMICAL DEFENSE				Total:17,573
<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>	
CHICKEN	40	PIG/SWINE	154	
FROG	112	RABBIT	374	
GUINEA PIG	1,427	RAT	2,166	
HAMSTER	11	SNAKE	2	
MOUSE	13,163	TOAD	95	
NON-HUMAN PRIMATE	29			

(M4) MEDICAL BIOLOGICAL DEFENSE				Total: 72,105
<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>	
GERBIL	30	MOUSE	67,748	
GOAT	2	NON-HUMAN PRIMATE	227	
GUINEA PIG	1,390	PIGEON	23	
HAMSTER	493	RABBIT	301	
HORSE	70	RAT	1,821	

(M5) HUMAN SYSTEMS TECHNOLOGY**Total: 7,436**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
CAT	3	NON-HUMAN PRIMATE	69
DOG	39	PIG/SWINE	146
FROG	265	RABBIT	77
GUINEA PIG	335	RAT	4,240
HAMSTER	994	SHEEP	97
MOUSE	1,171		

(M6) COMBAT CASUALTY CARE**Total: 19,224**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
DOG	15	NON-HUMAN PRIMATE	97
GOAT	53	PIG/SWINE	591
GUINEA PIG	301	RABBIT	1,111
HAMSTER	231	RAT	5,429
MOUSE	11,356	SHEEP	40

(M7) IONIZING RADIATION**Total: 6,033**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
DOG	10	MOUSE	3,960
FERRET	70	NON-HUMAN PRIMATE	100
GUINEA PIG	175	RAT	1,718

(M8) OTHER MEDICAL RDT&E**Total: 28,432**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
CAT	1	MOUSE	22,145
COW	16	NON-HUMAN PRIMATE	2
DOG	12	PIG/SWINE	4
FERRET	230	RABBIT	72
GOAT	3	RAT	4,668
GUINEA PIG	207	SALAMANDER	83
MINK	894	TOAD	95

(N1) PHYSICAL PROTECTION**Total: 1,039**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
MOUSE	97	RABBIT	16
NON-HUMAN PRIMATE	123	RAT	795
PIG/SWINE	8		

(N2) PHYSICAL DETECTION**Total: 193**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
CHICKEN	20	MOUSE	84
GOAT	18	RABBIT	71

(N3) OFFENSIVE WEAPONS TESTING**Total: 0****(N4) OTHER NON-MEDICAL RDT&E****Total: 39,713**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
BLUE WHALE	8	HUMPBACK WHALE	21
BOTTLENOSE DOLPHIN	15	KILLER WHALE (ORCA)	41
CALIFORNIA SEA LION	4	MINKE WHALE	1
CAT	38	MOUSE	4,931
DOLPHIN	11	NON-HUMAN PRIMATE	11
FALSE KILLER WHALE	2	NORTHERN ELEPHANT SEAL	1
FATHEAD MINNOW	130	PIG/SWINE	16
FERRET	20	PYGMY SPERM WHALE	1
FIN WHALE	10	RABBIT	329
FISH	2,125	RAINBOW TROUT	605
FROG	25,020	RAT	3,884
GERBIL	14	RIGHT WHALE	22
GRAY WHALE	8	SNAKE	67
GUINEA PIG	172	SONORAN TOPMINNOW	200
HAMSTER	800	WHITE WHALE	5
HARBOR SEAL	1	ZEBRA FISH	1,200

(C1) CLINICAL MEDICINE**Total: 18,105**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
DOG	9	NON-HUMAN PRIMATE	30
FERRET	97	OPOSSUM	1
GERBIL	75	PIG/SWINE	708
GOAT	7	PIGEON	15
GUINEA PIG	300	RABBIT	258
LAMB	1	RAT	7,823
MOUSE	7,581	TADPOLE	1,200

(C2) CLINICAL SURGERY**Total: 1,859**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
CHINCHILLA	143	MOUSE	275
DOG	61	NON-HUMAN PRIMATE	10
FERRET	42	PIG/SWINE	111
GOAT	135	RABBIT	396
GUINEA PIG	9	RAT	616
HAMSTER	48	SHEEP	13

(C3) OTHER CLINICAL INVESTIGATIONS**Total: 0****(T1) TRAINING, EDUCATION, AND/OR INSTRUCTION****Total: 6,680**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
CALF	2	HAMSTER	10
CAT	36	MOUSE	566
CHICKEN	20	NON-HUMAN PRIMATE	53
DOG	116	PIG/SWINE	840
FERRET	101	RABBIT	152
FROG	18	RAT	1,609
GERBIL	4	SHEEP	7
GOAT	3,028	SNAKE	13
GUINEA PIG	49	TOAD	56

(T2) OTHER TRAINING/INSTRUCTIONAL**Total: 0****(A1) ADJUNCTS TO ANIMAL USE RESEARCH****Total: 168**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
BELUGA WHALE	10	NON-HUMAN PRIMATE	8
BOTTLENOSE DOLPHIN	48	RAT	100
FALSE KILLER WHALE	1	RISSO'S DOLPHIN	1

(A2) ALTERNATIVES TO ANIMAL INVESTIGATION**Total: 10,899**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
AFRICAN CLAWED FROG	240	JAPANESE MEDAKA	8,600
BELUGA WHALE	2	RABBIT	12
BLUEGILL SUNFISH	400	RISSO'S DOLPHIN	1
BOTTLENOSE DOLPHIN	4	ZEBRA DANIO JAPANESE MEDAKA	1,640

(S) CLASSIFIED SECRET OR ABOVE**Total: 104**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
GOAT	37	PIG/SWINE	4
NON-HUMAN PRIMATE	40	RAT	23

(B) BREEDING STOCK**Total: 155**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
NON-HUMAN PRIMATE	139	RABBIT	16

(O) OTHER ANIMAL USE PURPOSES**Total: 1,310**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
BLUEGILL SUNFISH	800	GUINEA PIG	34
CHICKEN	1	MOUSE	33
FATHEAD MINNOW	400	RABBIT	15
GOOSE	5	RAT	22

GRAND TOTAL ANIMAL USE/RESEARCH**318,800**

(S) CLASSIFIED SECRET OR ABOVE**Total: 104**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
GOAT	37	PIG/SWINE	4
NON-HUMAN PRIMATE	40	RAT	23

(B) BREEDING STOCK**Total: 155**

<i>Species</i>	<i>Animals Used</i>	<i>Species</i>	<i>Animals Used</i>
NON-HUMAN PRIMATE	139	RABBIT	16

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GOOSE	5	RAT	22

GRAND TOTAL ANIMAL USE/RESEARCH**318,800**

Appendix P

Alternatives

Appendix P

Alternatives

Reduction - Decreasing the numbers 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.

Substitution of Another Species

- The best vaccine candidate was selected from rodent screening thus reducing the number of nonhuman primates required.
- Less chickens are needed than mammals for antibody production since a laying hen can produce almost 10 times more antibody than a rabbit over a similar time period.

Substitution of Computer Simulations or Other Technologies

- Historical controls in data analysis reduce the number of nonhuman primates used.
- Computer modeling for drug design, enzyme assay screens, and in vitro/ex vivo chemoprotective agent screens is a systemic approach that reduces the number of drugs required to evaluate the in vivo mouse model and reduces mouse use by >90%.
- Toxin and toxoid preparations are titrated in a cell assay to minimize the use of animals for dose determinations.
- Simultaneous evaluation of multiple experimental groups against a single set of controls for both aerosol and oral challenges reduces the number of animals required.
- The number of animals in the microvascular surgery course was reduced by the addition of suture boards and a "rubber rat" vascular model.
- In research on the harvested and reharvested central third of the patellar tendon, animals serve as their own control.
- Computer models reduce the number of animals required to evaluate blood vessels and tissues.
- CMR is a refined component of the whole cell causing no side effects, thus less test animals are required.
- Non-animal training aids reduce the number of animals required.
- PCR techniques for mRNA vasopressin allows the amplification of the signal so that even very small amounts of mRNA can be detected and less animals are used.
- The sheeps head minnow protocol uses an improved water quality system engineered for use with well water that has increased fish survival, thus reducing the numbers of required stock animals.

Reduction (cont.)

Substitution of Computer Simulations or Other Technologies (cont.)

- Western blot analysis of nitric oxide synthase (NOS) isoforms and PCR analysis of NOS mRNA allow for replacement of some studies utilizing pharmacologic tools in the whole animal.
- Molecular biological techniques to assess endothelins and receptor subtypes replaces the need to do all characterization of hormone and receptor subclassifications with pharmacologic tools in the whole animal.
- Multiple physiological assessments in the same animal allow for reduction in the total number of animals.

Sharing Animals between Research Investigations

- Sharing of animal tissues with other investigators reduces animal use.
- Animals assigned to previously conducted protocols that were vaccinated against or exposed to *B. anthracis* obviates the need for duplicating the vaccination and exposure of naive rhesus macaques.
- Hamsters fed upon by mosquitoes that are determined to be uninfected during transmission trials will be reused in another study under this protocol.
- Many different questions can be answered in the same experiment with aliquots of the tissue whereas only one condition can be investigated using the animal.
- Coordination of obtaining renal tissue for molecular biological assessments from rats used for other protocols reduces the total number of animals used throughout the research program.

Refinement - The refinement alternative for animal use addresses the need to ensure that the maximum humane use of each animal is obtained through proper protocol design and efficient utilization of animals, or through the modification of the experimental design to reduce the ethical cost associated with the study.

Reduce Pain

- Animals which have produced sufficient ascitic fluid from the antigen/adjuvant combination alone, prior to day 28 will not be given sarcoma cells, thus alleviating unnecessary pain as an effect of the tumor cell growth.
- The less reactogenic ribi adjuvant system is utilized over classical adjuvants.
- Anesthesia is administered prior to taking blood samples, thus limiting trauma to the animals.
- Preparation of viral antigens in the rabbit kidney cell line with rabbit serum or serum-free media method reduces the immunological response by the host, thus lessening the adverse reactions.
- Early intervention adjustment to the endpoint during toxicity testing phase will prevent unnecessary pain and suffering to symptomatic animals.
- Monkeys receive only one intramuscular immunization, thus the systemic antibody response is anticipated to be very low.
- Knowledge of the disease process in the animal model allows for sick animals to be accessed more accurately and to be euthanized prior to unnecessary pain and suffering.
- Buprenorphine provides analgesia for clinically ill animals and the relative illness system for early identification of euthanasia candidates is utilized, thus minimizing pain and discomfort.
- Currently available analgesics provide a maximum of 8-12 hours of pain control, thus greatly increasing ability to provide analgesia with a minimum of manipulation and stress to the animal.
- The oral vaccine preparation is a refinement compared to the parenteral route.
- Denervation and local anesthetics to rabbit ears prevent pain in a partial thickness ear burn during the time of model development.
- Postoperative analgesia for relief of potential edge pain is utilized in most full thickness burn protocols using rats.
- Use of local anesthetic in rabbits reduces postoperative pain after surgical implantation of catheters and flow probes.
- Ability to sample via in dwelling catheters avoids animal discomfort as urine and blood samples are obtained.

Refinement (cont.)

Reduce Pain (cont.)

- Subcutaneous port placement eliminates repeat episodes of blood withdrawal and drug administration from rabbits in studies to evaluate antimalarial drugs.
- Acute studies done under continuous anesthesia avoid discomfort during physiologic measurements.
- Endpoint of euthanasia, before the onset of severe clinical signs of renal failure, prevents pain and suffering.
- An osmotic pump for the delivery of bromodeoxyuridine instead of administering multiple injections to the rats reduces pain.

Reduce Distress

- The subcutaneous hormone pellets provide steady serum estradiol levels and precludes the need for daily injections in rats.
- The recovery of antibodies from eggs is non-invasive, thus animal bleeding is not required.
- Utilization of the environmental enrichment strategy, including toys, constitutes a refinement.
- Implantable temperature transponders is a refinement over invasive rectal thermometers.
- Rabbits are provided with enrichment toys.
- Cows, horses, burros, sheep, and goats are acclimated and trained to lead by a halter or collar (geese are trained with a hand restraint), thus minimizing prephlebotomy stress.
- Tympanic thermometry is less invasive and decreases the length of time an animal must be restrained to measure body temperature.
- The blood volume amount is refined from 10% of the body weight to 6%, thus placing less stress on the animals.
- Animals are housed in social settings (i.e., pairs or groups) in an enriched environment (e.g., nestboxes, toys).
- The anesthetic periods are for restraint alone and scheduled to maximize the number of procedures done per animal per episode.
- The use of radiotelemetry is a refinement by lowering the stress-induced artifact (e.g., anesthesia), and by providing long, continuous periods of data collection.
- Continuous monitoring of physiological parameters by telemetry in an unrestrained animal with the use of nonlethal endpoints constitutes a refinement over the standard SEB - rhesus monkey model.
- Anesthetics are administered to reduce distress in mice that are to be bled.

Refinement (cont.)

Research Models and Animal Alternatives

- Plethysmography measures minute volumes prior to aerosol exposure and allows for better assessment of the inhaled dose.
- Animals are exsanguinated after anesthesia to perform research on their blood and/or tissue.
- Initial training utilizes inanimate training aids thus providing students with a basic level of skill to enable better skills with live tissue.
- Identification and control of infectious diseases research have adapted several mosquito colonies to membrane feeding, thus guinea pigs and rabbits are not exposed directly to mosquitoes.
- Pilot studies to refine techniques and define the animal model are utilized so that animal use can be kept to the minimum required for statistical significance.
- Live vaccine mutants are evaluated in macrophage tissue culture for the ability to infect cells and survive within cells before being considered for animal inoculation.
- Wantanabe Heritable Hyperlipemic (WHHL) rabbits obviates the need for cholesterol feeding and balloon injury since these rabbits develop spontaneous hypercholesterolemia and have identified atherosclerotic plaques by three months of age.
- With fluoroscopy, IV stents can be placed in both iliac arteries, which is well tolerated by the animal and bilateral femoral artery cut down is unnecessary.
- During laproscopic training, students practice before attempting the procedures on an anesthetized animal.

Replacement - The replacement alternative addresses supplanting animal use with non-living systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. Additionally, human subjects are used when experimental drugs and other procedures progress to human trials. Such trials are conducted in accordance with Title 32, U.S. Code of Federal Regulations, Section 219, "Protection of Human Subjects in DoD-Sponsored Research."

Non-mammalian Species or Species Lower in the Phylogenetic Scale

- Purifying antibodies from the egg yolk of hyperimmunized chickens are a replacement to antibodies raised in the serum of mammals.
- Advances in the production and use of insect and vertebrate cell lines have reduced the need for intracerebral and intraperitoneal inoculation of suckling mice for the isolation of arboviruses.
- Research on a new insect repellent uses mice instead of nonhuman primates.
- Use of mice replaces rhesus monkeys required in research on immune response to recombinant dengue virus proteins.
- The South African clawed frog (*Xenopus laevis*) embryo replaces laboratory mammals commonly used in teratogenesis assays.
- Various freshwater fishes replace mammals commonly used in toxicology research.
- Various freshwater fishes replace laboratory rodents commonly used in cancer research.
- Single-cell invertebrates replace higher animals used in toxicity testing.
- Free-ranging honeybee is used in place of animal sentinels to monitor contaminated environments.
- A rabbit model is used to evaluate antimalarial drugs instead of the nonhuman primate model.

Biochemical/ Physical Methods

- The hydra assay is a screening test used to detect potential developmental toxicity associated with exposure to chemical compounds which replaces vertebrate animal testing.
- Chicken ovalbumin is used as a test antigen to verify immunological tolerance when selecting and map testing vaccine candidates.

Computer Simulations

- Rat toxicology screen has been replaced with a computer model.

Discarded Tissue from Other Laboratories or Re-use of Animals

- Porcine hearts from a local slaughterhouse are used to practice mitral valve replacement surgery.

Replacement (cont.)

Other Species Replace Companion Animals

- Pigs have replaced dogs in various training protocols.
- Goats have replaced dogs in the advanced trauma life support provider course and in vascular surgery techniques.
- Ferrets have replaced cats in the pediatric advanced life support course and in endotracheal intubation exercises.

Appendix Q

Letters from Dr. Martin Stephens



The Humane Society of the United States
2100 L Street, N.W.
Washington, D.C. 20037
(202) 452-1100
FAX (202) 778-6132

February 7, 1992

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K. William Wiseman

Dr. Harry Salem
U.S. Army CRDEC
SMCCR-RST
Aberdeen Proving Ground, MD 21010

Dear Harry:

Congratulations on organizing what was clearly a successful conference on alternatives. What was particularly heartening from my perspective was all the new faces I had not seen before on the alternatives "circuit." We need that new blood and diversity.

If you are organizing another conference on alternatives, and could use a speaker from an animal protection organization, just let me know. I would be happy to oblige.

Again, congratulations.

Best wishes,

Martin L. Stephens, Ph.D.
Vice President
Laboratory Animals

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June 1, 1994

Harry Salem, Ph.D.
Edgewood Research, Development & Engineering Center
Attn: SCBRD-RTL
U.S. Army
Aberdeen Proving Ground, Maryland 21010-5423

Dear Harry:

Congratulations on organizing another successful conference on alternative methods for safety testing. I appreciated the opportunity to participate in the session on oral, ocular, and dermal irritation.

You and I discussed tracking down some of the military's historical data on human eye irritation. These data are based on clinical studies that were apparently conducted at the Aberdeen Proving Ground many years ago. Given the importance attached to good human data at the conference, I think the military could do the alternatives community a big service by locating these data and assessing their value in evaluating alternative methods of eye irritation assessment. This project could also help the military fulfill its congressional mandate to advance the field of alternatives.

Let me know what you think.

Best wishes.

Sincerely,

Martin L. Stephens, Ph.D.
Vice President
Laboratory Animal Issues

The Humane Society of the United States
2100 L Street, NW, Washington, DC 20037
(202) 452-1100 FAX (202) 778-6132

Appendix R

Food and Drug Administration Group Recognition Award



**Food and
Drug
Administration**

Group Recognition Award

PRESENTED TO

Harry Salen

*as a member of Interagency Regulatory Alternatives
Group (IRAG)*

*For outstanding contribution in facilitating the reduction, refinement and replacement
of animal testing, and advancing the development of non-whole animal alternative
methods.*

May 9, 1997

DATE

Michael A. Friedman

LEAD DEPUTY COMMISSIONER
FOOD AND DRUG ADMINISTRATION