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

on

Department of Defense Animal Care and Use Programs 1997

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List of Acronyms

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
AALAS	American Association of Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
APHIS	Animal and Plant Health Inspection Service
ASBREM	Armed Services Biomedical Research Evaluation and Management
AWA	Animal Welfare Act
AWIC	Animal Welfare Information Center
BRD	Biomedical Research Database
CNS	Central Nervous System
CRISP	Computer Retrieval Information on Scientific Projects
DDR&E	Director, Defense Research and Engineering
DoD	Department of Defense
DTIC	Defense Technical Information Center
FDA	Food and Drug Administration
FEDRIP	Federal Research in Progress
FY	Fiscal Year
IACUC	Institutional Animal Care and Use Committee
IG	Inspector General
ILAR	Institute of Laboratory Animal Research
IRAG	Interagency Regulatory Alternatives Group
JDL	Joint Directors of Laboratories
JTCG	Joint Technology Coordinating Groups
LAM	Laboratory Animal Medicine
MATRIS	Manpower and Training Research Information Services
NIH	National Institutes of Health
NMR	Nuclear Magnetic Resonance
NOS	Nitric Oxide Synthase
NRC	National Research Council
OPRR	Office for the Protection from Research Risks
OSD	Office of the Secretary of Defense
PCR	Polymerase Chain Reaction
PHS	Public Health Service
POC	Point of Contact (Primary Contact)
RDT&E	Research, Development, Test, and Evaluation
S&T	Science and Technology
STO	Science and Technology Objective
TAPSTEM	Training and Personnel Systems Science and Technology Evaluation and Management
USAMRMC	United States Army Medical Research and Materiel Command
USDA	United States Department of Agriculture
VEE	Venezuelan Equine Encephalitis
WRAIR	Walter Reed Army Institute of Research

SECTION I
INTRODUCTION/OVERVIEW

This is the Fiscal Year (FY) 1997 Report to Congress on Department of Defense Animal Care and Use Programs. In addition to a general overview, this report provides a detailed accounting of Department of Defense (DoD) animal use; to include its publicly accessible database, animal care and use oversight procedures, Institutional Animal Care and Use Committees (IACUCs), alternatives to animal use programs, Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) status, and animal use.

The report covers animal research conducted by the DoD including education, training, and testing both in DoD laboratories and by extramural projects funded by the Department for FY97. This report does not include information on animals used by the DoD solely for the purpose of food preparation for human or animal consumption, ceremonial activities, recreation, or the training, care, and use of military working animals.

I.1 REQUIREMENTS FOR USE OF ANIMALS IN THE DOD

Department of Defense use of animals in research, development, education, and training is critical to sustained technological superiority in military operations in defense of our national interests. The DoD's biomedical research, development, test, and evaluation (RDT&E) and training programs that are dependent on animal use ultimately translate into improved military readiness as well as reduction in morbidity and mortality associated with military operations. These programs contribute directly to ensuring that service men and women maximize their capabilities to survive the numerous and various hazards they face around the world. Additionally, many examples of the humanitarian benefits of the DoD investment in animal research that are shared on an international basis improve the quality of life of both humans and animals. Several prime examples of the humanitarian benefits of DoD research efforts are: the Junin vaccine that has provided critical protection for more than 120,000 individuals in endemic areas of Argentina against the ravages of Argentinian hemorrhagic fever; DoD-developed Venezuelan equine encephalitis (VEE), eastern equine encephalitis, and western equine encephalitis vaccines that have been used to limit and control epidemics of VEE in Venezuela and Colombia in 1995, and to protect occupational workers in vaccine production plants around the world. In addition to being important public health tools, the equine encephalitides vaccines are obviously critical adjuncts to animal health programs around the world.

Biomedical research has benefited greatly from animal use alternatives such as non-living systems, cell and tissue culture, and computer technology. However, complex human organ systems interactions, in addition to environmental factors and confounding variables, necessitate the continued judicious use of animal models in DoD programs. Although many innovative animal use alternatives have been developed and are in use by Department scientists, situations remain in which there are no acceptable non-animal alternatives available. As new advances, technologies and breakthroughs in animal use alternatives occur, the DoD will embrace them whenever possible. The chapter on alternatives in this report gives a full accounting of the aggressive programs and numerous animal use alternatives implemented in DoD laboratories.

Disease remains a major cause of death and disability in military operations and conflicts. During Operations Desert Storm and Restore Hope, outbreaks of respiratory diseases, diarrheal diseases such as shigellosis, and parasitic diseases such as leishmaniasis and malaria, threatened the health and well-being of our troops. Indeed, the DoD is still assessing and addressing concerns over the long-term effects of various environmental, physical, and medical factors associated with the Persian Gulf Conflict. It is obvious that the health

and well-being of military personnel extend far beyond the immediate scope of the battlefield. We have an irrefutable moral obligation to our soldiers, sailors, airmen, and marines to provide the maximum protection and care possible. DoD researchers are committed to accomplishing this goal, and in many cases, animal-based research is the critical underpinning for the fulfillment of that obligation.

The DoD must develop the materiel and technological means to best protect and sustain the health and well-being of service men and women against all threats, and provide the best medical treatment possible to those who become casualties. This responsibility underlies the need for the DoD to conduct research, and to train and educate military health-care providers in the most effective medical management of battlefield casualties. Battlefield health care must very often be provided in an austere, harsh and hostile environment, hours away from a definitive care hospital, unlike medical counterparts found in civilian emergency medicine and trauma management. A domestic, low velocity projectile gunshot patient in a modern civilian shock and trauma center will be supported and resuscitated by a full complement of medical staff with a plentiful supply of oxygen, fluids, medications, surgical intervention and nursing. The combat casualty may be supported by only a single aidman and the medical supplies, experience, and expertise he can carry.

One of the most critical areas requiring DoD animal use is the compelling need to develop vaccines, drugs, and therapies to protect, sustain and treat service men and women during military operations. These research programs are strongly focused on a myriad of militarily relevant diseases and threats, many of which can result in potentially fatal diseases or conditions that have no known treatments, therapies, or cures. Consequently, there are numerous instances, including medical chemical and biological warfare defense, where animal-based studies are particularly critical. Ethical concerns, as well as regulatory requirements of the Food and Drug Administration (FDA), necessitate that candidate vaccines and drugs be safe and efficacious in laboratory animal models prior to initiation of human use protocols whenever possible. The rationale for this is to prevent the fielding and use of ineffective or dangerous treatments. Indeed, during the final stages of vaccine and drug development, large-scale safety and efficacy testing is usually conducted using human volunteers. However, in the search for understanding and developing protection against many highly lethal agents, human use protocols are simply not possible. Consequently, carefully regulated animal use is absolutely vital to the success of Department biomedical research programs. The ultimate goal is to maximize the survivability of our troops in all situations.

I.2 DoD Policy Governing Animal Research

The Department of Defense is committed to full ethical and regulatory compliance for its animal-based biomedical research programs. DoD has been proactive in increasing the fixed infrastructure and span of control necessary to ensure lawful and efficient execution of programs and maximize oversight of diverse and varied missions. The Department has aggressively implemented focused programs and working documents that optimize standardization of animal care and use at the user level. This enhanced standardization and oversight have improved a historically good system, and made it outstanding.

In 1995, the DoD revised and implemented the directive dealing specifically with animal care and use (DoD Directive 3216.1, "The Use of Animals in DoD Programs," 1995) (Appendix A). This directive strengthens and clarifies requirements for nonaffiliated membership on IACUCs and directs all DoD animal use facilities that maintain animals for research, testing and training to apply for AAALAC accreditation.

The DoD also implemented a Policy Memorandum entitled "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" (Appendix B). This 1995 policy letter specifies training requirements for nonaffiliated DoD IACUC members and implements a standard format for animal use protocols (Appendix C), a standard checklist for IACUC inspections (Appendix D), and a standard reporting requirement for all animal use research to support a publicly accessible database (Section II).

All animal research must conform to requirements of the 1966 Animal Welfare Act (P.L. 89-544) as amended in 1970 (P.L. 91-579), 1976 (P.L. 94-279) and 1985 (P.L. 99-198), as well as the National Research Council *Guide for the Care and Use of Laboratory Animals*, (7th rev. edition, 1996), U.S. Government Principles for Animal Use (1985) (Appendix E), and the requirements of the applicable regulations of the United States Department of Agriculture (USDA).

Although the Animal Welfare Act currently exempts mice and rats in the genera *Mus* and *Rattus*, the DoD has long afforded them, along with all other vertebrates, the same consideration given non-exempt species under the Animal Welfare Act. At the same time, DoD biomedical researchers have aggressively developed novel procedures to replace, reduce, and refine the use of animals during experimentation.

I.3 Benefits of Animal Research

DoD's laboratories and extramural contractors provide the capability to solve the medical and non-medical problems of the future through the efforts of internationally renowned medical and scientific experts working in state-of-the-art facilities and in the field. The Department conducts or funds research, development, training and evaluation to sustain the operational capabilities of today's service men and women. As noted in the previous section, many of these programs require the use of animals to meet their mission requirements. These programs result in many benefits for both the military and civilian sector (Table I-1). The military benefits from programs that do research in areas that currently threaten military personnel such as combat trauma, chemical and biological agents, infectious diseases not endemic to the United States, directed energy, and occupationally unique health hazards from military operations and environmental extremes. These research programs focus heavily on the prevention of casualties; these efforts contribute significantly to the readiness and sustainment of the DoD's warfighting capability, and also to a significant reduction in the number of casualties reaching the medical treatment facilities. In addition, the DoD is involved in medical research that directly benefits the civilian population such as research in breast cancer, cardiovascular disease, trauma care and treatment, respiratory injuries, burns, and specific surgical procedures. A list of specific benefits by research category is shown at Appendix F.

***Table I-1** Animal Use Benefits*

Medical
- FDA safety and efficacy testing prior to use in humans
- Vaccine development
- Participation in Phases 2, 3, and 4 drug evaluations
- Gulf War illnesses
- Breast cancer research
- Identification and control of insect vectors of infectious disease
- Understanding of decompression and predict susceptibility to oxygen seizures
- Development of new techniques for mechanically assisting respiration
- Establish potential hazards of military nerve agents to humans
- Prevention and diagnosis of human Schistosomiasis
- Detection of visual impairments caused by high G loads
- Identification of medical countermeasures for botulinum toxin
- Identification of an antiviral drug as a potential therapy for filoviruses such as Ebola
- Development and testing of a unique bandage utilizing fibrin glue for dramatic control of massive hemorrhage
- Development of models of acute lung injury that mimic neonatal and adult disorders
- Development of drugs that enhance acquisition of information and prolong retention of memory

Clinical
- Improvement in patient care
- Bridging the gap between science and bedside treatment
- Better understanding of general anesthetics during surgery

Non-Medical
- Development of biosensors
- Identification of environmental toxins

Training
- Special forces medical training
- Advanced trauma life support training
- Graduate medical training in surgical techniques

Alternatives
- Development of alternatives to replace, reduce, and refine the use of animals

Besides the medical benefits of animal research there are many other non-medical and training benefits. The development of biosensors and the identification of environmental toxins benefit both the military and civilian communities. The DoD has many exceptional medical and scientific educational programs that train both medical personnel and scientists. While these people are in the military, the DoD reaps the benefit of this training; once they leave the military, this benefit is realized by the civilian community. The development of alternatives to animal use by the DoD provides an extra value to both communities and to animals as they discover ways to reduce or replace the use of animals. Also refinement research results in more humane methods of performing research that is applied in many types of research settings.

I.4 Scope of Report

This report provides a comprehensive accounting of DoD biomedical research and animal care and use programs. There are sections that include in-depth discussions of:

a. Publicly accessible information on Department research (Section II),

b. Policies and procedures for oversight of Department animal care and use programs (Section III),

c. AAALAC accreditation for Department animal care and use programs (Section IV),

d. DoD animal use profiles (Section V), and

e. DoD initiatives to promote alternative methods that replace, reduce, or refine animal use (Section VI).

I.4.1 Publicly Accessible Information on Animal Use in the DoD

On October 1, 1995, the Department of Defense implemented a publicly accessible database analogous to the National Institutes of Health Computer Retrieval of Information on Scientific Projects System. The DoD Biomedical Research Database (BRD) is available online to the public, and is composed of succinct summaries of Department research projects, allowing interested individuals easy access to Department research information. The cost of animal-based research is presented by work unit summary in the BRD. In order to prevent duplication, this information is not presented in this report. More information on accessing the database is presented in Section II.

I.4.2 Oversight of DoD Animal Care and Use Programs

DoD animal use oversight is reviewed in Section III. In general, internal and external oversight provisions for animal research conducted by the DoD are at least as stringent as those for research in any other department of the federal government, and in many ways exceed the standards. As a matter of policy, the DoD abides by the applicable federal regulations pertaining to animal care and use, including provisions for oversight. All DoD facilities and extramural institutions sponsored by the DoD must submit proposals for animal use to an IACUC. The IACUCs review proposed animal protocols to ensure compliance with the Animal Welfare Act, and address concerns of the community. The DoD Directive 3216.1 (1995) establishes oversight requirements that exceed the provisions of the Animal Welfare Act. Each IACUC serves as an independent decision-making body for the institution and establishes policy for the care and use of animals at that facility in accordance with applicable DoD directives, federal law and regulations.

The DoD has developed and implemented a standardized protocol format for use by all of its units (Appendix C). It includes requirements for search of Federal Research in Progress database or an equivalent database and the Defense Technical Information Center database to prevent duplication of ongoing federally funded research. The principal investigator must justify the use of animals, including consideration of alternatives, justify the choice of species and the number of subjects, and include a literature search and assurance that the work does not needlessly duplicate prior experimentation. The protocol must specify procedures to be used with animals, methods to avoid or minimize pain, include a literature search for possible alternatives, qualifications of the

individuals conducting procedures with animals, and disposition of animals at the termination of the work.

The IACUC ensures that personnel involved in animal-based studies are properly trained and, if necessary, establishes a training program to support the staff. The IACUC inspects facilities and animal care programs at least twice annually, and prepares a written report including a plan to address deficiencies. It enforces compliance with procedures specified in the protocols by conducting inspections, evaluating and, if necessary, investigating reports of deviation from approved procedures. The IACUC of each facility performs semiannual program reviews of all animal use areas. The DoD 1995 Policy Letter strengthens that process by establishing a standardized semiannual review checklist that outlines the areas required for IACUC review. This guidance is consistent with the recommendations of the DoD Inspector General (IG) report of February 1994 (Appendix G). A formal report of inspection shall be prepared twice annually, noting the use of the checklist, and indicating all major and minor deficiencies, a plan for correction of deficiencies, signatures of a majority of IACUC members, and a statement indicating whether there are or are not minority opinions. Finally, the IACUC serves as an impartial investigator of reports of violations of good animal practices and is empowered to suspend the use of animals for protocols not conducted in accordance with the Animal Welfare Act or institutional policy.

DoD Directive 3216.1 (revised in 1995) clarifies composition, membership, and training requirements of the IACUC. The 1995 changes address the House Armed Services Committee's request to improve community representation and to appoint animal advocates to the Department's IACUCs, consistent with a recommendation of the IG Report of February 1994. The revised Directive (1995) increases the minimum membership of all DoD IACUCs from three to five. In addition, it specifies that

> "there shall be at least one non-scientific member on the IACUC. In addition, there shall be at least one member representing the general community interest who is nonaffiliated with the research facility. The nonaffiliated member 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 nonaffiliated member shall be designated for all IACUCs having a single nonaffiliated membership."

Each DoD IACUC has increased its membership to comply with this Directive.

This Directive exceeds the requirements of the Animal Welfare Act and is further strengthened by the DoD 1995 Policy Letter which requires a minimum of 8 hours of training for new non-affiliated members. In support of this training, the DoD developed a program consisting of a set of topics and recommended resources that may be used by individual IACUCs.

Responsibility for oversight of the Department's science and technology programs rests with the Director, Defense Research and Engineering (DDR&E). The staff, in conjunction with representatives from the Services, annually review the science and technology efforts to ensure they are fully coordinated and without unnecessary duplication of effort. The preponderance of animal use within the Department occurs in biomedical programs. These activities receive specific oversight from the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee, which was created by congressional direction in 1981. The ASBREM Committee is chaired by the DDR&E and co-chaired by the Assistant Secretary of Defense (Health Affairs). The overall biomedical effort is carefully integrated and reviewed to eliminate unjustified duplication of effort by seven subordinate Joint Technology Coordinating Groups reporting to the co-chairpersons.

I.4.3 Accreditation of DoD Laboratories by AAALAC

Animal use programs in the DoD strive to meet all the requirements of AAALAC. AAALAC accreditation is recognized as the "Gold Standard" for animal care and use programs. DoD Directive 3216.1 (1995) states that all DoD laboratories that maintain animals for use in research, testing or

training shall apply for AAALAC accreditation. Currently there are 34 DoD animal facilities worldwide, of these 33 (97%) are accredited.

Over the past 5 years, the DoD has been resolute in pursuing AAALAC accreditation for all of the facilities that use animals in research. This diligence has resulted in an increase in accreditation from 60% in 1993 to 97% today.

I.4.4 DoD Animal Use Profiles by Research Category

A profile of DoD animal use is provided in Section V. In this report, a detailed system was adopted for classifying animal use that includes 8 categories with 23 subcategories: 8 medical research, 4 non-medical research, 3 clinical research, 2 training, and 6 other categories of studies and use. Detailed charts and graphs are included in Section V.

In 1997, the DoD used 316,048 animals, which is a 1% decrease from FY96. Of these, 22,014 (7%) were USDA reportable species as defined in the Animal Welfare Act of 1985. Table I-2 summarizes the major animal use statistics for DoD research. In addition, it should be noted that no animals were used for development or testing of offensive weapons. During the time that the DoD has been reporting animal use to Congress (1993-1997), there has been a 43% decrease in the total number of animals used.

I.4.5 DoD Initiatives to Promote Alternative Methods that Replace, Reduce, and Refine the Use of Animals

Congress requested that the DoD establish aggressive programs to replace, reduce, and refine current use of animals. A review of DoD programs and initiatives to develop and implement alternatives to animal research is reviewed in Section VI. Alternatives presented are those developed by DoD investigators and the general and specific alternatives implemented by the DoD in 1997.

Animal research is an essential part of the scientific process, but it is only initiated after due consideration of alternatives. The DoD uses a

Table I-2 *Summary of DoD Animal Use Statistics*

Total Animal Use by Species	% of Total
Rodents, fish, amphibians and birds	97.21
Rabbits	0.86
Farm animals (i.e., sheep, pigs, cows, horses)	1.11
Dogs, cats, nonhuman primates, marine mammals	0.71
Other	0.12

Percentages may not add up to 100% due to rounding of calculations.

Total Animal Use by Category	% of Use
Medical RDT&E	77.95
Non-Medical RDT&E	5.31
Clinical Investigation	10.13
Adjuncts/Alternatives	3.82
Training & Instructional	1.17
Breeding Stock	0.02
Classified Secret or Above	0.20
Other	1.40

Percentages may not add up to 100% due to rounding of calculations.

Standard Protocol Format that specifically requires each investigator to consider alternatives to the use of animals and to justify the animal model selected. In addition, all protocols that involve unrelieved pain or discomfort require consultation with a veterinarian prior to IACUC review, and a specific database search for scientifically acceptable alternatives to the proposed method. Each protocol that involves animals in research or training must explain the need for the animal research and defend the choice of species as the most scientifically valid model. Often, economies of time and resources are gained when scientifically valid alternatives to animal use are available. Our review of current animal research reveals that scientists in the DoD

have developed or adopted many alternative methods based on ethical considerations and other inherent benefits. Table I-3 presents examples of alternatives developed by the Department in FY97 to replace, reduce and refine the use of animals. In addition, the Department sponsors conferences and workshops to promote alternatives to animal research. The DoD has funded the Institute of Laboratory Animal Research (ILAR) of the National Research Council to develop institutional training materials, education, and publications in support of DoD laboratory animal care and use programs since 1987. The Department has resolved to maintain this important collaboration by providing in excess of $100,000 annually for the ILAR Program. The IACUC process also includes a strong emphasis on consideration of alternatives in all new protocols.

In conclusion, it is the policy of the DoD that animal utilization will be conducted in full compliance with the Animal Welfare Act and that animals are used in research only when scientifically acceptable alternatives are not available. At the same time, the use of animals in research is essential to protect the health and lives of military personnel; therefore, the DoD will be engaged in biomedical research that involves the use of animals for the foreseeable future.

***Table I-3** Examples of Alternatives for Replacement, Reduction, and Refinement of the Animals Developed or Being Developed by the DoD*

- The DoD is developing environmental enrichment programs for nonhuman primates and ferrets.
- Artificial human skin is used to study inflammatory responses to heat and medical countermeasures against vesicant agents.
- DoD investigators have developed an artificial eye with lenses that can mimic the focusing characteristics of the eye.
- A technique was developed to examine individual neurons in culture, eliminating the need for intracranial surgery.
- Using cell culture assay for initial determination of antiviral activity greatly reduces (>90%) the number of compounds requiring testing in animals.
- Realistic biophysical models computationally simulate the damage processes induced by lasers as accurately as possible.
- Isolated perfused porcine skin flap technique generates more precise data, thus reducing the number of experimental replicates (animals) needed to obtain data on the dermal adsorption of chemicals.

SECTION II

PUBLICLY ACCESSIBLE INFORMATION ON ANIMAL USE IN THE DOD

II.1 CONGRESSIONAL REQUEST INFORMATION

House Armed Services Committee Report 4301 (1995) requested the Secretary of Defense to "develop a mechanism for providing Congress and interested constituents with timely information... about [Department of Defense (DoD)] animal use programs, projects and activities, both intramural and extramural." In response to this request, and to serve the interest of both the scientific community and general public, the Department has implemented a publicly accessible database called the Department of Defense Biomedical Research Database (BRD). The BRD is a database containing succinct summaries of the Department's research projects involving the use of animals. This database is analogous to the National Institutes of Health (NIH) Computer Retrieval of Information on Scientific Projects (CRISP) System. The CRISP System is a biomedical database containing information on research projects supported by the United States Public Health Service, as well as information on intramural research programs of the NIH and the Food and Drug Administration. The BRD became accessible to the public through the Internet on October 1, 1995. It is located on the Manpower and Training Research Information Services (MATRIS) home page.

II.2 THE FY96 BIOMEDICAL RESEARCH DATABASE

The data in the FY96 BRD were developed from the current work unit summary system of the Defense Technical Information Center (DTIC). DoD organizations performing research, development, test and evaluation (RDT&E) projects are currently mandated to provide annual reports of research to the DTIC. The DTIC maintains these work unit summaries in a database. While the majority of DoD animal use occurs in RDT&E projects, some work is performed in clinical investigations programs that are not mandated to provide work unit summaries to the DTIC. Therefore, the DoD directed that these non-RDT&E DoD animal research projects develop summaries to be entered into the BRD. The areas of research, testing and training in the FY96 BRD include, but are not limited to, the following: infectious diseases, biological hazards, toxicology, medical chemical defense, medical biological defense, clinical medicine, clinical surgery, physical protection, training, graduate medical education and instruction.

Military activities that house, care, or use animals provided a work unit summary for any animal-based research. The FY96 BRD contained summaries and was made accessible to the public on October 1, 1997. A work unit summary may refer to a single protocol or a series of protocols that are performed in a given category of animal use. The summaries include the following information:

Title: Title of the work unit.

Funding Fiscal Year: The funding for the entire work for a given fiscal year. The funding includes civilian salaries, cost of animals, cost of materials, cost of human-based research, cost of non-animal based research, etc. - all costs related to the work unit except military salaries.

POC/Author: The primary contact (POC) for the work unit is usually the Public Affairs Office.

POC Address: The complete mailing address of the POC.

Performing Organization: The name of the activity where the work is performed.

Objective and Approach: This section is a narrative on the objectives and the approach of the work unit. This narrative provides a general summary of the work.

Indexing Terms (Descriptors): A list of indexing terms or keywords. The keywords

contain "animals" and the term for any animal types which may be used in the work unit (e.g., guinea pigs, rats).

These summaries were compiled into the BRD and organized into a presentation format for the Internet.

II.3 Access and Use of the Biomedical Research Database

The BRD can be accessed at:

http://dticam.dtic.mil/dodbr

The BRD home page shown in Figure II-1 is a searchable database. To perform a search, click on Search. This will bring up the DoD BRD search page. The database can be searched by title, keywords, description or specific demographic fields (Figure II-2). The results of the search will produce a hypertext list of titles (Figure II-3). To access a particular summary, click on the specific title and the summary will appear (Figure II-4).

II.4 FY97 Update of the Biomedical Research Database

The DoD will make all FY97 work unit summaries of animal use in research, testing, education, and training available to the public this year. All military activities that house, care, and/or use animals have provided summary information on any animal research, testing, education, or training work for the FY97 BRD. The cost of FY97 animal-based research is presented by work unit summary in the BRD. In order to prevent duplication, this information is not presented in this report. These data will become available to the public on October 1, 1998.

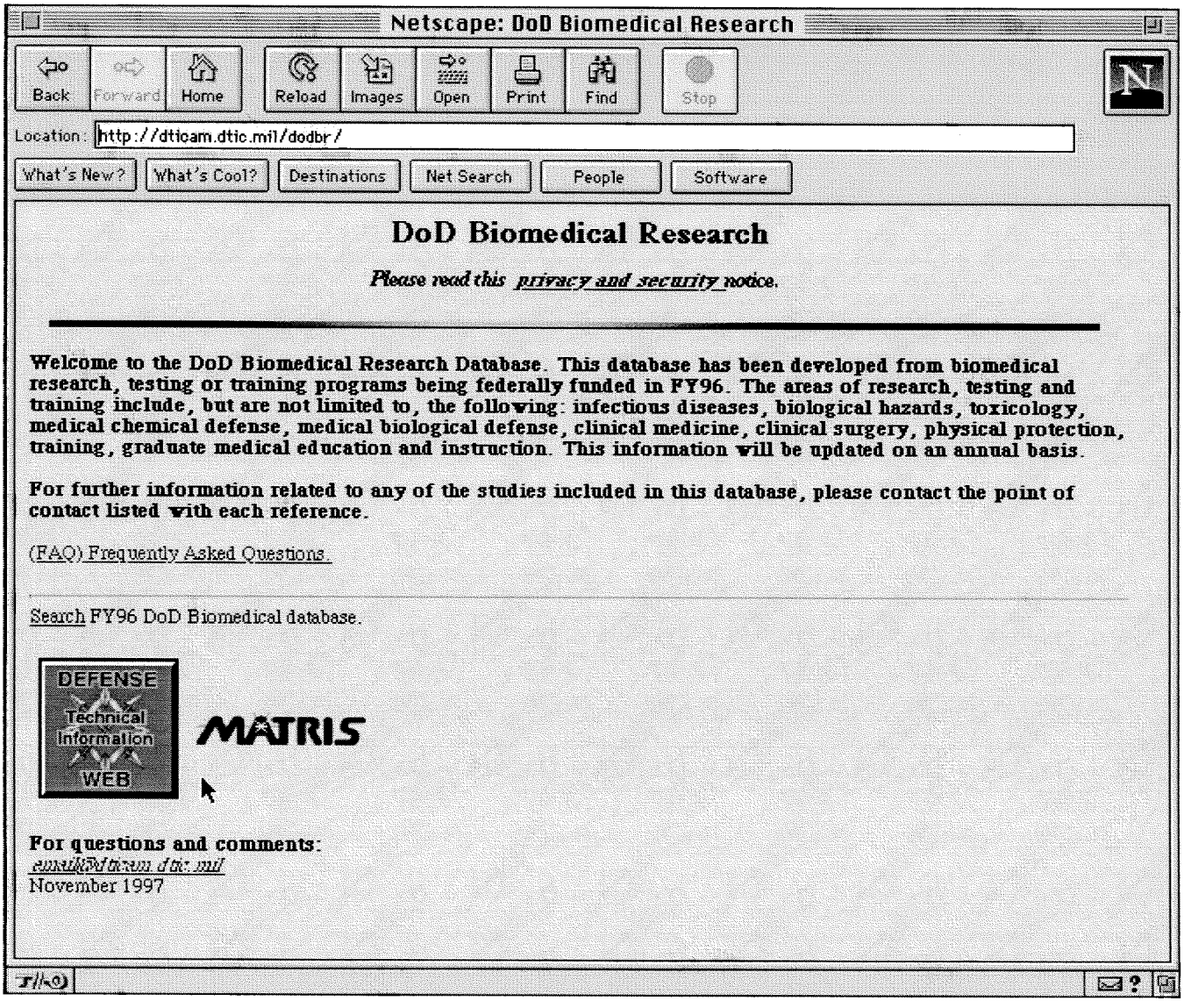


Figure II-1 DoD Biomedical Research Database Home Page

Netscape: <MATRIS.WWW>BIOWWW Search Form

Back | Forward | Home | Reload | Images | Open | Print | Find | Stop

Location: http://ocean.dtic.mil/BASIS/matris/www/biowww/sf

What's New? | What's Cool? | Destinations | Net Search | People | Software

BASIS | Search | Terms | Help

DoD BIOMEDICAL DATABASE

Start Search | Clear

Title :
contains the phrase

Keyword(s) :
contains the phrase Toxicology

Description :
contains the phrase

Fields : PI State
contains the phrase

Order By: NONE **Sort Order:** Ascend **Field Connector:** OR

Start Search | Clear

BASIS | Search | Terms | Help

Figure II-2 DoD BRD Search Page

Netscape: <MATRIS.WWW>BIOWWW Documents 1 to 20 of 20

Back | Forward | Home | Reload | Images | Open | Print | Find | Stop

Location: http://ocean.dtic.mil/BASIS/matris/www/biowww/SDF

What's New? | What's Cool? | Destinations | Net Search | People | Software

BASIS | Search | Terms | Summary | Expand | Down Load | Prior | Top | Up | Down | Bottom | Next | Help

DoD BIOMEDICAL DATABASE

Documents	Title
1	Toxicity Test Model R&D
2	Evaluation of Biomonitoring Systems for Assessment of Contaminated Waters and Sediments at U.S. Army Installations
3	Acute Inhalation Toxicity of Neutralized GB in Rats by the Munitions Management Device, Version 1 (MMD-1) Wastestream Process
4	Preclinical Toxicology Studies of New Drugs and Vaccines
5	Perform Preclinical Toxicology Studies of New Drugs
6	Screening of Air Force Chemicals
7	Toxicology and Human Health Issues
8	Molecular Mechanisms of Toxicity
9	Hydrocarbon Remediation Issues
10	Species Differences in Skin Penetration
11	Regulation of Metallothionein Gene Expression
12	Reference Dose Development for JP-4 Jet Fuel
13	Persian Gulf Veterans Health Effects Research
14	Biologically Based Pharmacokinetic (PBPK) Models of Water-Soluble Organic Acid
15	Modeling of Retinoic Acid-Induced Limb Dysmorphogenesis
16	Assessment of the Toxicity of SFE, A Fire Extinguishant and Potential Substitute for Ozone Depleting Substances in Environments of Potential Military Interest
17	Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors
18	Health Effects of Imbedded Depleted Uranium
19	Behavioral Assessment of Neurotoxicities Associated with Dideoxynucleoside Administration
20	Detoxication Enzymes as Biomarkers of Toxin Exposure

BASIS | Search | Terms | Summary | Expand | Down Load | Prior | Top | Up | Down | Bottom | Next | Help

Figure II-3 *Search Results on Toxicology from the BRD*

Netscape: <MATRIS.WWW>BIOWWW Document 13 of 20

Back | Forward | Home | Reload | Images | Open | Print | Find | Stop

Location: http://ocean.dtic.mil/BASIS/matris/www/biowww/DDW?W%3DKEYWORDS%20PH%20IS%20%27Toxicology%27%2

What's New? | What's Cool? | Destinations | Net Search | People | Software

BASIS | Search | Terms | Summary | Expand | Down Load | Prior | Top | Up | Down | Bottom | Next | Help

DoD BIOMEDICAL DATABASE

Title: Persian Gulf Veterans Health Effects Research
Keyword(s): animal;rat;chemical stressors;Persian Gulf War;physiological changes;**toxicology**
Current Funding Year: 96 **Current Funding Amount**: 95000

PI Org: Armstrong Laboratory / Toxicology Division
PI Code: OL AL HSC / OET
PI Address: 2856 G Street
PI City: Wright-Patterson Air Force Base **PI State**: OH **PI Zip**: 45433-7400
PI Phone: 513-255-3423 **PI DSN**: 785-3423

RI Org: Armstrong Laboratory HSC/PA
RI Name: Public Affairs Office
RI City: Brooks AFB **RI State**: TX **RI Zip**: 78235-5118
RI Phone: (210) 536-3234 **RI DSN**: 240-3234

Description:

Objective: To test the research hypothesis that simultaneous exposure to a combination of chemical stressors, each well tolerated individually, causes subtle physiological changes when coupled with psychological stress. This simulates a potential exposure scenario during the Persian Gulf War.

Approach: Exposed Persian Gulf Veterans may have subtle physiological changes from exposure during the Persian Gulf War which render them more susceptible to Persian Gulf Veterans' Illnesses (PGVI) than similar unexposed, non-deployed individuals. Exposure conditions leading to development of these physiological changes will be simulated and a laboratory animal model will be exposed to these conditions. The effects of such exposures on behavioral, cognitive, and neuromuscular performance; and neurotransmitter levels will be evaluated and compared to control animals. In addition, induction of stress proteins will be measured; a biomarker of effect or exposure may be identified during this research.

BASIS | Search | Terms | Summary | Expand | Down Load | Prior | Top | Up | Down | Bottom | Next | Help

Figure II-4 *Sample of Publicly Accessible Summary*

SECTION III
OVERSIGHT OF DoD ANIMAL CARE AND USE PROGRAMS

This section of the Department of Defense (DoD) Report to Congress provides a detailed overview of the formal mechanisms and strategies for providing adequate oversight to the Department's numerous animal care and use programs. For the purposes of this report, research is defined as those congressionally authorized science and technology (S&T)-based activities—Title II, Research, Development, Test and Evaluation—of the Military Departments for which funds are appropriated within program elements 6.1 (Basic Research), 6.2 (Exploratory Development) and 6.3 (Advanced Development).

The mechanisms detailed here show a clear and long-standing commitment by the DoD to manage its biomedical research and clinical programs in a systematic, comprehensive, and effective manner. Individual programs are driven by specific mission requirements, and are subjected to a thorough, stratified review and analysis prior to commitment of funds. The DoD uses animals only when necessary to complete its mission, and in full compliance with applicable laws, regulations, and guidelines.

III.1 DETERMINATION OF DoD NEEDS FOR ANIMAL RESEARCH

Determining research needs and plans is a comprehensive process integrated into the DoD's planning, programming, and budgeting mechanisms. Integral elements of these processes are the Department's Research and Development Descriptive Summaries submitted to Congress in justification of the annual budget request. These summaries provide the Office of the Secretary of Defense, the Office of Management and Budget, and Congress with significant detail concerning the accomplishments and future plans of every research project.

Each DoD research laboratory employs its available resources to tailor its organization, staffing, and related infrastructure to best meet its S&T mission and to support the accountability, responsibility, and authority of its commander. In October 1995, the Department implemented a comprehensive DoD Standard Protocol Format as a basis to justify and document all proposed animal use (Appendix C). The Standard Protocol Format solicits specific information that ensures a thorough review of all animal use proposals by Institutional Animal Care and Use Committees (IACUCs). Although there are minor differences in specific procedural elements in protocol review procedures among DoD facilities, DoD regulations ensure that the overall review mechanisms remain fundamentally similar. The general submission, review, and approval processes are summarized here.

An investigator develops a research protocol in support of Departmental S&T guidance and other supplementing instructions developed within the chain of command, both external and internal to the laboratory. Augmenting the formal S&T coordination and review process is a literature search to verify nonduplication of previous or ongoing research. The Standard Protocol Format requires that a search of Federal Research in Progress (FEDRIP), or its equivalent, and the Defense Technical Information Center (DTIC) database be made for DoD-funded research. An additional search of the scientific literature (MEDLINE, GRATEFUL MED, MEDLARS, AWIC, etc.) is highly recommended. Review and certification that this requirement has been met are integral elements of the review and approval process prior to initiation of a research project.

If animal use is planned for the intended research, the principal investigator must prepare an animal protocol request for submission to the facility IACUC. In addition to the DTIC and FEDRIP search, the Standard Protocol Format requires detailed information regarding results and dates of other on-line database searches (e.g., AWIC, AGRICOLA, CAAT, MEDLINE) that may yield alternatives to painful procedures. Additional

pertinent knowledge and information on the proposed study are gained through review of the scientific literature and participation in scientific meetings, symposia, and workshops detailing other ongoing or completed research.

Since protocols employ DoD resources, individual protocols are reviewed for factors such as military relevance, necessity, scientific merit, and relative research priority. These reviews are normally conducted within the laboratory's command-and-control structure and are characterized by the features of peer review systems.

DoD IACUCs carefully review research proposals involving the care and use of animals for numerous factors, including but not limited to ensuring that (a) the study is based on sound scientific principles; (b) a minimum number of animals are used to achieve the purpose; (c) the lowest phylogenetic species is selected as the appropriate model; (d) there is appropriate use of analgesics and anesthetics or, if required, there is adequate scientific justification for not using anesthetics; (e) the research is not duplicative; (f) the research personnel have the training and experience needed to conduct the research; and (g) the scientific question is of sufficient importance to warrant the use of animals. Additionally, detailed information regarding methodology, techniques, schedules, etc., is required, greatly facilitating a comprehensive and thorough review by IACUCs.

III.2 Oversight of Animal Care and Use Programs and Facilities

There are three principal vehicles for oversight of animal care and use programs at DoD research facilities: Major DoD Activities and Service Command Staff, the local IACUC, and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

III.2.1 Military Departments

Each military department has one or more components responsible for oversight and review of its research facilities and animal care and use programs. Periodic reviews, site visits, and inspections are conducted formally, and reports are prepared as required.

The Army's ultimate oversight responsibility is divided between two major commands: the U.S. Army Medical Command and the U.S. Army Materiel Command. In the U.S. Army Medical Command, programmatic guidance and site visits are performed by specialty trained laboratory animal medicine (LAM) veterinarians in the Headquarters, U.S. Army Medical Research and Materiel Command, and the U.S. Army Medical Department Center and School (Veterinary Programs Manager). In the U.S. Army Materiel Command, oversight is provided by a specialty trained LAM veterinarian assigned to the U.S. Army Chemical and Biological Defense Command.

Ultimate responsibility for laboratory animal care and use in the Navy is divided between the Office of the Chief of Naval Research and the Office of the Surgeon General of the Navy. Oversight for both offices is accomplished by a specialty trained LAM veterinarian assigned to the Naval Bureau of Medicine and Surgery and attached for duty with the Naval Medical Research and Development Command. Besides biomedical research oversight, this LAM veterinarian also serves the Naval School of Health Sciences, Bethesda (Clinical Investigations) and the Inspector General at the Naval Bureau of Medicine and Surgery.

III.2.2 IACUCs

The backbone of the review process for all DoD animal-based research is the IACUC review of the research proposal or protocol. DoD Directive 3216.1, "The Use of Animals in DoD Programs," requires all DoD facilities using animals in research to comply with the Animal Welfare Act (AWA). The AWA requires the Chief Executive Officer to appoint an IACUC, qualified through the experience and expertise of its members, to assess the research facility's animal program, facilities, and procedures. The AWA requires that IACUCs have a minimum of three members: an appropriately qualified chairman, at least one member not affiliated with the institution in any way other than as a member of the Committee, and a veterinarian with training or experience in laboratory animal medicine and science. Each DoD IACUC is chaired by an individual with credentials and experience appropriate to the post, typically a senior physician, scientist, or veterinarian. DoD Directive 3216.1 (1995) (Appendix A) clarifies the composition,

membership, and training requirements of the IACUC. This Directive increases the minimum size of all DoD IACUCs from three to five, which is in concert with the National Institutes of Health (NIH) Office for Protection from Research Risks (OPRR) model. In addition, it specifies that

> "...there shall be at least one non-scientific member on the IACUC. In addition, there shall be at least one member representing the general community interest who is nonaffiliated with the research facility. The nonaffiliated member 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 nonaffiliated member shall be designated for all IACUCs having a single nonaffiliated membership."

The 34 IACUC panels reporting in FY97 averaged just over eight members each. Private civilian, government civilian, and military representation on the panels is 9%, 47%, and 44%, respectively.

The diverse backgrounds/professions of the nonaffiliated and alternate nonaffiliated IACUC members are provided in Appendix H. Currently, 53% of the nonaffiliated members are private sector civilians, the remainder are federal government civilians or military personnel. In accordance with Directive 3216.1, these members represent the community and are not affiliated with (not under the command of) the research facility. Full compliance with the Directive has resulted in an increase in the overall number of DoD IACUC members.

This Directive exceeds the requirements of the AWA and is further strengthened by the DoD 1995 Policy Letter (Appendix B) that directs a minimum of 8 hours of training for the new nonaffiliated members. DoD IACUCs implemented these requirements October 1, 1995. All DoD new nonaffiliated IACUC members received at least 8 hours of training to fulfill the requirement. The total hours of training reported for nonaffiliated, FY97 IACUC panel members averaged 11.8.

Each IACUC has at least one Doctor of Veterinary Medicine with training or experience in laboratory animal science and medicine who serves as an animal advocate. The U.S. Army Veterinary Corps' formal postgraduate training program in laboratory animal medicine provides didactic training in IACUC composition, function, and regulatory requirements. This training also prepares them to serve as animal advocates. Of the 34 reporting DoD institutions, 14 had 2 or more veterinarians serving on their IACUC panel.

It is a proactive Department policy that nonaffiliated members participate fully in discussions and vote on all research proposals. They are also encouraged to perform unannounced site visits of animal care facilities. In FY97 nonaffiliated members made at least 28 unannounced visits to Department animal facilities.

The IACUC has statutory responsibility for reviewing the facility's animal care and use program and inspecting the animal facilities on a semiannual basis. Consequently, at least once every 6 months, each IACUC performs an in-depth review of the animal care and use program and inspects the animal facilities. To facilitate these inspections, the DoD has developed and implemented a standardized semiannual program review checklist that details the requirements of the review. All DoD IACUCs are currently using the new standardized checklist during their semiannual program reviews. The IACUCs prepare written reports of their evaluations and submit them to the Institutional Official, usually the facility commander. Reports specifically address compliance with the AWA, identify any departures from the Act, and include an explanation for the departure. The report must distinguish between major and minor deficiencies and provide a schedule for resolution of deficiencies.

All DoD IACUCs document their meetings and activities, including the results of inspections, complaints, actions, and training. They are empowered to review and investigate concerns involving the care and use of animals at the research facility resulting from complaints received from the public or in-house workers, or from reports of noncompliance received from laboratory personnel. To facilitate the reporting and resolution of complaints or concerns, facilities commonly place signs or notices in high-traffic areas and in animal-study areas advising both the public and personnel

who work with animals how to contact members of the IACUC, facility commanders, and/or the Inspector General (IG) whenever questions arise concerning humane care and treatment of animals. Among the reporting DoD institutions, three complaints were registered during FY97. DoD facilities have developed a wide variety of proactive and innovative mechanisms to inform the public on how to contact responsible individuals and to ensure that those who work with animals are fully apprised of the requirement to provide humane and ethical care (Appendix I). Additionally, IACUCs make recommendations to the Institutional Official regarding any aspect of the research facility, its animal program, or the training of its personnel; review and approve, require modification to, or withhold approval of new research protocols involving the use of animals; review and approve, require modification to, or withhold approval of proposed significant changes regarding the care and use of animals in ongoing research protocols; and suspend an activity involving animals when they determine that the activity is not being conducted in accordance with its approved protocol.

III.2.3 AAALAC

AAALAC is a nonprofit organization chartered to promote high quality standards of animal care, use, and welfare through the accreditation process.

The AAALAC accreditation process provides scientists and administrators with an independent, rigorous assessment of the organization's animal care and use program. To increase accountability and tracking, a centralized DoD point of contact and database for AAALAC information have been established to enhance monitoring, reporting, and facilitation of the AAALAC accreditation process. An in-depth discussion of the AAALAC accreditation process and a profile of the DoD's participation are provided in Section IV.

III.2.4 Training

The DoD provides extensive veterinary and animal care services for its facilities. Veterinarians with specialty training in LAM direct programs for animal care and use throughout the Department. They serve as a valuable resource to the research staff and the IACUC to ensure that all research methods and maintenance procedures are consistent with the latest principles of animal medicine, and with the current interpretations and implementing regulations of the AWA. The DoD sponsors formal postdoctoral training programs for veterinarians in LAM, including a nationally recognized, in-house 2-year residency program culminating in specialty board eligibility for certification in the American College of Laboratory Animal Medicine. Many DoD veterinarians attend various university postgraduate LAM training programs resulting in a master's degree in public health or Ph.D. It is significant that approximately 25% of the current membership of American College of Laboratory Animal Medicine, the veterinary specialty most closely associated with animal welfare and laboratory animal care and use, received either all or part of their training in DoD-sponsored LAM training programs. In August 1995, the DoD began a formal postgraduate Master's of Public Health in Laboratory Animal Medicine at the Uniformed Services University of the Health Sciences. This outstanding program provides the Department with a new source of LAM experts who will significantly enhance animal welfare in our research laboratories.

In addition to veterinarians, the DoD trains animal care specialists (Military Occupation Specialty 91T) to assist in the daily management, care, and treatment of laboratory animals. Over the last 30 years, the DoD has trained over 3,600 animal care specialists. Since 1986, the Division of Veterinary Medicine has sponsored the Walter Reed Army Institute of Research (WRAIR) DoD Laboratory Animal Workshop program. Many of the workshops focus on species-specific techniques and handling, while others provide general laboratory animal information required by federal law and other guidelines for the research mission. Successful completion of the workshops fulfills the training requirements for use of those animals in research protocols. The WRAIR DoD Laboratory Animal Workshop FY97 schedule is provided in Appendix J. Additionally, DoD research institutions send appropriate staff to a variety of seminars and workshops sponsored by the National Institutes of Health, other federal agencies, and private institutions dedicated to the proper care and use of research animals. The Annual Public Responsibility in Medicine and Research Meeting is an outstanding example of this type of training.

The DoD provides detailed informational and instructional material to all members of the

IACUC, including nonaffiliated members, to ensure that they are fully cognizant of the numerous responsibilities of IACUC members under the provisions of the AWA. DoD Directive 3216.1 "The Use of Animals in DoD Programs" requires new nonaffiliated IACUC members to receive an initial 8 hours of training and continued training for IACUC members, investigators, and technicians. This requirement went into effect October 1, 1995. Although training is an individual institute's responsibility, the DoD has developed a program consisting of a set of topics and recommended resources to support the training requirement (Appendix K). The topics are meant to be general and allow for tailoring of the training to meet the institute's specific needs. The recommended resources are readily available commercially. Formal training on animal care and use issues is provided to all appropriate personnel in Department research laboratories in accordance with the provisions of the AWA. Examples of training or materials currently provided to IACUC members are detailed in Appendix K. One of the examples listed in Appendix K is the Institute of Laboratory Animal Research (ILAR) publication *Education and Training in the Care and Use of Laboratory Animals*. As one of the major sponsors of this publication, the DoD has established a formal relationship with the National Research Council (NRC), an extension of the National Academy of Sciences. The publication is used as a guide by the DoD and has been translated into five languages. Many countries use this publication as a standard for the care and use of laboratory animals.

III.2.5 Community Visits

Individuals or groups wishing to visit Department facilities need to comply with certain procedural guidelines. All DoD facilities are served by a public affairs office, at either the facility, post, or base. Visits by the public or the press are arranged and coordinated through the appropriate public affairs office. While most facilities described few community visits, two institutions reported hosting over 50 such visits in FY97. DoD facilities are visited by various special interest groups including community and civic groups; animal welfare or animal advocates, groups, or individuals; dignitaries, academia, and teachers; local, state, and national politicians; congressional members and staff; elementary to postdoctoral students; etc. Consequently, a greatly diversified range of individuals is constantly visiting and observing the quality of Department facilities.

III.2.6 Office for Protection from Research Risk Oversight

A number of DoD research laboratories participate in the NIH grants process. Institutional compliance with The Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) is a prerequisite for granting or continuation of NIH intramural and extramural funding. The formal vehicle for compliance with the PHS Policy is an "Animal Welfare Assurance" negotiated between individual institutions and the OPRR. The principal references for the negotiation of an OPRR "assurance" are the Health Research Extension Act of 1985 (Public Law 99-158, November 20, 1985, "Animals in Research"), the Animal Welfare Act, and NRC's *Guide for the Care and Use of Laboratory Animals*. Consequently, OPRR provides additional oversight to those laboratories that have negotiated OPRR assurances.

III.2.7 Additional Oversight

Within the DoD, individuals may raise animal welfare concerns. This may be with the IACUC, facility commanders, the IG, or the attending veterinarian. Other means of compliance or concern may be voiced through "Waste, Fraud and Abuse Hotlines," or the formal chain of command. Procedures to enhance and facilitate these mechanisms have been implemented in DoD facilities.

The function of the IACUC and the role of an ombudsman are augmented by the Department's IG. An ombudsman is defined by Webster's dictionary as "a government official charged with investigating citizens' complaints against the government." The Humane Society of the United States, a witness at the April 7, 1992 hearing on The Use of Animals in Research by the Department of Defense before the House Armed Services Committee, offered the Ombudsman Program at the Massachusetts Institute of Technology as an example of a model program. This program consists of an ombudsman assigned to the university president's office to hear complaints regardless of the nature. These include personnel complaints, sexual harassment, animal welfare, etc.

The DoD assigns this responsibility to its IG and respective Inspectors General of the Military Departments. In addition, military bases and large organizations on military bases have their own Inspectors General who fulfill this function. Significantly, complaints to IG can be made anonymously. Also of note is the fact that IG investigations are conducted with complete autonomy, and are completely insulated and immune to pressure from the chain of command.

Oversight of extramural (contract) animal-based research is provided for in DoD Directive 3216.1 (1995). It states 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 contract facilities performing DoD-sponsored research using nonhuman 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."

As directed by DoD Directive 3216.1, all nonhuman primate protocols receive an additional centralized review external to the research facility.

III.3 Chain of Command over Animal Care and Use Programs

The chain of command is designed to resolve problems at the lowest possible level. It provides control and communication among various components of organizations. Each link in the chain of command is a level of responsibility and authority that extends from the President of the United States, as Commander in Chief, down to the lowest supervisory level. Different levels within the chain have different responsibilities and authority. Each level in the chain is responsible for a lower level and accountable to a higher one. Every individual in the military is part of the chain of command and is accountable to it.

III.4 Avoidance of Unintended Duplication of Research

Both the DoD and Congress have a long history of concern about the potential for unintended duplication of Defense research. Within the past decade, the Department has initiated significant improvements in its mechanisms for coordination, and joint planning and review of its research programs.

In 1981, Congress expressed concerns about the potential for unnecessary duplication of biomedical research among the Military Departments (H.R. 96-1317). This resulted in the DoD proposing an Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee to coordinate biomedical research planning and the conduct of biomedical research among the Military Departments. Congress fully endorsed and built upon this proposal by establishing DoD Lead Agencies for major elements of the biomedical research programs for which there were either no, or very few, service-unique requirements (H.R. 97-332). For example, the Army was designated the DoD Lead Agency for military infectious disease and combat maxillofacial research while the Navy was designated DoD Lead Agency for preventive and emergency dentistry research. The ASBREM Committee established Joint Technology Coordinating Groups (JTCGs), consisting of directors of biomedical research programs and representatives of biomedical research laboratories, to coordinate all DoD biomedical research planning and execution. The ASBREM Committee process

has proven to be highly effective at eliminating unnecessary duplication of biomedical research.

The ASBREM Committee process became the model for joint DoD coordination initiatives. Responsibility for joint coordination, planning, execution, and review of the Department's S&T programs was assigned to joint oversight bodies: the Joint Directors of Laboratories (JDL), the ASBREM Committee, the Training and Personnel Systems Science and Technology Evaluation and Management (TAPSTEM) Committee, and the Joint Engineers. The resulting technology area responsibilities are shown in Figure III-1. Joint S&T oversight bodies are assisted in execution of their responsibilities by subordinate S&T coordinating groups that are focused on coordination of specific technology areas. For example, the ASBREM Committee is supported by the JTCGs (Figure III-2), and the JDL is supported by separate technology panels.

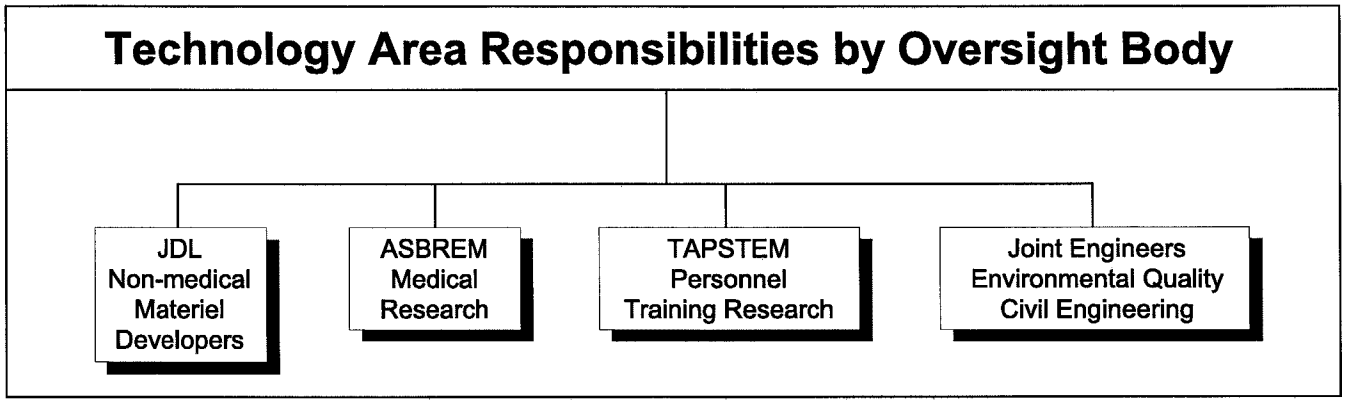


Figure III-1 DoD Technology Area Responsibilities

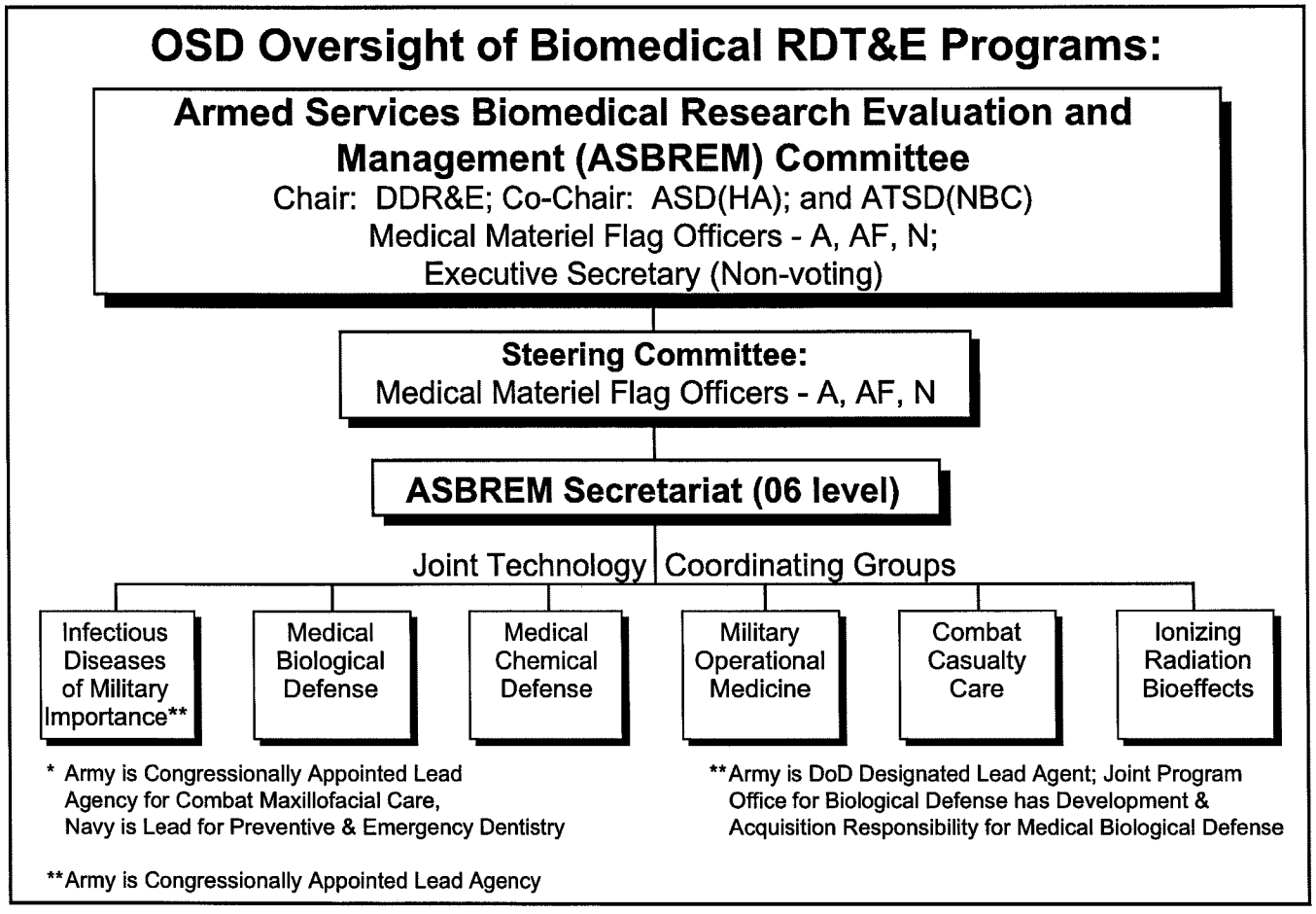


Figure III-2 Structure of ASBREM Committee

In addition to these formal coordination and review processes to eliminate research duplication, there are a number of less formal mechanisms that provide significant disincentives for research duplication. Competition, both in-house and extramural, for research support is a prominent feature of S&T; each year large numbers of scientifically meritorious research proposals cannot be funded due to shrinking resources and funding shortages. In most cases the professional stature of individual scientists or engineers among their peers is measured by their individual and original contributions to the scientific literature. There is little if any reward for unnecessarily duplicating the work of others; such actions often have significant negative impact on how the scientist or engineer is viewed by peers and on the ability to secure research support. Additionally, within the DoD civilian personnel system, scientists' and engineers' pay grades are determined in part by the level of individual scientific and technological contributions. One outcome of research is publication of a manuscript in a professional journal. A sample listing of journals with DoD animal research publications is found in Appendix L. Peer-reviewed journals critique the research during the review process, leading to an overall enhancement of the research process and to validation of both the scientific merit and necessity of the research. These less formal, relatively unquantifiable, disincentives substantially augment and buttress the Department's formal mechanisms for regulating and avoiding unnecessary research duplication within its S&T programs.

III.5 Avoidance of Unnecessary Research

The same factors that effectively prevent unwarranted duplication of research are applied to prevent unnecessary research. Additionally, through Cooperative Research and Development Agreements, the Department has increased its emphasis on leveraging and exploiting, for Defense needs, S&T investments from other federal agencies, U.S. industry, and academic institutions, and the international scientific community. Past descriptions of Defense S&T "spin-off" have been supplanted by programs intended to "spin-on" accomplishments by others as well as to optimize the dual-use potential of the Defense S&T investment. The foundation of Defense S&T strategy is the application of S&T accomplishments to sustain Defense technological superiority through efficient and responsive modernization of our warfighting capabilities.

III.6 Summary

Biomedical research using animals is highly structured and regulated in the United States, being governed by numerous laws, regulations, and policies. Consequently, the DoD has a number of stratified formal and informal mechanisms for reviewing, regulating, and executing its biomedical research mission and animal care and use programs. Research performed by the DoD receives close programmatic, scientific, and regulatory scrutiny, being carefully reviewed by various offices, committees, and program managers before it is funded or implemented. These reviews serve to determine the necessity to the mission, provide oversight of animal care and use, and avoid unnecessary or unintended duplication of research.

Individual IACUCs provide oversight of animal care and use programs and research. They also provide training and information about animal care and use, and ensure the humane use of animals in research. Each DoD facility's IG is also an effective means for investigation of concerns about the necessity of animal use, as well as the ethical treatment and humane care of animals used in DoD research.

Over the past decade, the DoD, in concert with Congress, has streamlined and greatly improved coordination of its S&T activities to avoid unnecessary duplication and provide a focused program of research responsive to the DoD's unique and wide-ranging needs.

When viewed in its totality, the Department's significant progress and investment in administration, infrastructure, standardization, training, and oversight of animal use are indeed impressive, and can serve as useful models for the rest of the biomedical research community.

SECTION IV
AAALAC ACCREDITATION OF DoD LABORATORIES

The Department of Defense (DoD) recognizes the benefits of accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). With the publication of the Joint Regulation on the Use of Animals in DoD programs, June 1, 1984 (AR 70-18), the DoD implemented more stringent animal care and use requirements than those required by statute. The Joint Regulation established uniform procedures, policies, and responsibilities for the use of animals in the DoD. The DoD has elevated the requirement with the current DoD Directive 3216.1 (1995), which states that "all DoD laboratories that maintain animals for use in research, testing or training shall apply for AAALAC accreditation." The Joint Service Regulation also cites the National Research Council (NRC) publication, *Guide for the Care and Use of Laboratory Animals,* which is the principal document used by AAALAC in its accreditation process. The animal care and husbandry standards and requirements contained in the Guide are designed to provide an environment that ensures proper care and humane treatment are given to all animals used in research, testing, and training. This care requires scientific and professional judgment based on knowledge of the husbandry needs of each species, as well as the special requirements of the research program.

IV.1 AAALAC ACCREDITATION

AAALAC accreditation is widely accepted by the scientific community, and viewed as an extremely desirable feature of the Department's animal care and use programs. The Association is highly respected as an independent organization that evaluates the quality of laboratory animal care and use. Accreditation covers all aspects of animal care to include institutional policies; laboratory animal husbandry; veterinary care; facility physical plant; support facilities; and special areas of breeding colony operations and animal research involving hazardous agents such as radioactive substances, infectious agents, or toxic chemicals.

The independent and external peer review that is fundamental to continuing AAALAC accreditation is valuable to any program. All AAALAC findings highlight program strengths and identify potential weaknesses. Laboratories maintaining accreditation demonstrate a high degree of accountability and program excellence. AAALAC standards stress the appropriate appointment, composition, and empowerment of an Institutional Animal Care and Use Committee (IACUC). This Committee is responsible for monitoring and evaluating all aspects of the institution's program that uses animals for teaching and/or research purposes. IACUC functions are addressed in Section III of this report.

IV.2 DoD PROGRAM REVIEWS

The DoD utilizes external peer review by the Joint Commission for Accreditation of Health Organizations to evaluate many of its programs such as drug screening laboratories and military medical facilities. At the same time, the DoD recognizes the diversity of mission operations and global reach of the military mission. There are situations where external peer reviews are not cost effective due to remote locale, limited scope of operations, or host nation sovereignty. In these cases, equivalency standards can be applied and effectively monitored. The Joint Service Regulation and Service-conducted inspections of facilities implement the requirements of the Animal Welfare Act and the 1996 NRC *Guide for the Care and Use of Laboratory Animals.*

The DoD is committed to continuing its full participation in the AAALAC accreditation process in order to effect external peer review for assessing program compliance with regulations, guidance, and ethical responsibility.

IV.3 DoD AAALAC Accredited Programs

The number of DoD AAALAC accredited programs that maintain animals for research testing and training has significantly increased over the past 5 years (Figure IV-1). Of the 34 DoD facilities worldwide reporting animal use, 33 (97%) are AAALAC accredited. This percentage reflects the DoD's commitment to accrediting all of its animal care and use programs.

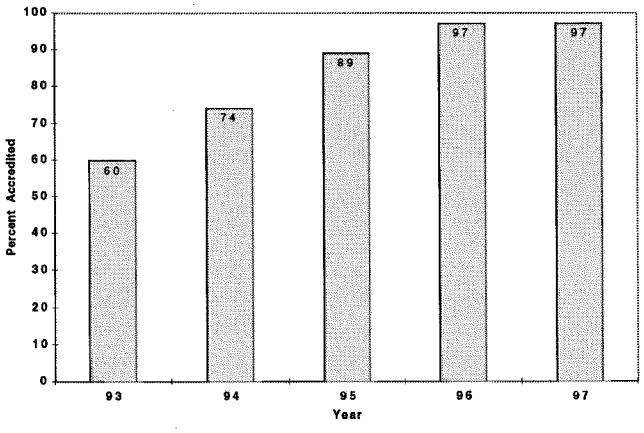


Figure IV-1 DoD AAALAC Accreditation at Time of Publication of the FY93-97 Reports

IV.4 AAALAC Accreditation Status for U.S. DoD Programs

There are 30 programs in the United States that maintain animals for research, testing, or training for the DoD. All programs in the U.S. are accredited by AAALAC. In addition, there are four DoD animal use programs that share DoD AAALAC accredited facilities. These programs are small detachments that are assigned to DoD bases and therefore share their animal care and use facilities. Appendix M provides additional information on AAALAC accreditation by program.

IV.5 AAALAC Accreditation Status for DoD Overseas Programs

There are four DoD programs using animals outside the United States. In foreign countries, the accreditation process is often complicated by issues of sovereignty; local governments have their own regulations and policies that must be considered. Renegotiation of various agreements may be involved in construction or renovation projects. Despite these and various other impediments, the DoD has raised the standard of excellence in its animal care and use programs by receiving full accreditation in three of its four overseas laboratories. The Naval Medical Research Detachment in Lima, Peru, was the first laboratory in South America to have received AAALAC accreditation. The Naval Medical Research Unit #2 in Jakarta, Indonesia, and the Naval Medical Research Unit #3 in Cairo, Egypt were the first to be accredited in Southeast Asia and Africa, respectively. The Armed Forces Research Institute of Medical Sciences in Thailand, which was being renovated during FY97, is the only non-AAALAC accredited facility.

SECTION V
DoD ANIMAL USE PROFILES

The information presented in this section provides profiles on the use of animals in various research categories, and the U.S. Department of Agriculture (USDA) pain categories of Department of Defense (DoD) animal-based research, testing and training programs for fiscal year (FY) 1997.

V.1 METHODS

Information was solicited and received from DoD agencies and military commands, organizations, and activities involved in animal care and use programs located both inside and outside of the United States. This included extramural contractors and grantees that performed animal-based research. For the purpose of this reporting requirement, an intramural program represents research performed at a DoD facility and funded by either DoD or non-DoD funds. An extramural program represents research performed by a contractor or grantee that is funded by the DoD.

V.1.1 Animal Use Profiles

The animal use profiles prepared for this report are consistent with the reporting information and data provided to the USDA using Animal and Plant Health Inspection Service (APHIS) Form 7023. In addition, this report contains comprehensive information on all other animals (e.g., mice, rats, birds) used that are not required in reports to the USDA.

For the purposes of this reporting requirement, an animal was defined as any whole nonhuman vertebrate, living or dead, excluding embryos, that was used for research, development, test, and evaluation (RDT&E), clinical investigations, diagnostic procedures, and/or instructional programs. Only live animals or whole dead animals, as defined, that were either on hand in the facility or acquired during FY97 and used are included. Animal organs, tissues, cells, blood, fluid components, and/or by-products purchased or acquired as such animal/biological components are not reported. This definition does not include animals used or intended for use as food for consumption by humans or animals, animals used for ceremonial purposes, or military working animals and their training programs.

A single animal was counted only once in determining the number of animals used during the fiscal year for a particular work unit or protocol. This does not refer to the number of times an individual animal was injected, manipulated, handled, or administered medication and/or experimental compounds within a given work unit, protocol, or program. Animals on hand during FY97, but not actually used during the fiscal year, are not included in this number.

V.1.2 Animal Use Categories

All DoD agencies and military commands, organizations, and activities involved in the performance and/or funding of animal care and use programs reported animal work by the category that best describes the general purpose of the animal use. If these categories did not describe the animal use within a particular work effort, the animal was placed under the Other category. The 8 general categories and 23 specific subcategories are listed in Table V-1. In-depth information on specific activities performed within a subcategory is presented in Appendix N. The medical research categories correspond to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee's Joint Technology Coordinating Group Medical Research Areas. Non-medical categories consist of RDT&E programs performed outside the ASBREM Committee medical oversight. Clinical Investigations studies were performed under the auspices of the Assistant Secretary of Defense for Health Affairs and the military services medical departments through Major Force Program 8 funding. These studies were usually in support of graduate medical education training programs located at the major military medical centers.

Table V-1 Animal Use Categories

MEDICAL (M)
- M1: Military Dentistry
- M2: Infectious Diseases
- M3: Medical Chemical Defense
- M4: Medical Biological Defense
- M5: Human Systems Technology
- M6: Combat Casualty Care
- M7: Ionizing Radiation
- M8: Other Medical RDT&E

NON-MEDICAL (N)
- N1: Physical Protection
- N2: Physical Detection
- N3: Offensive Weapons Testing
- N4: Other Non-Medical RDT&E

CLINICAL INVESTIGATIONS (C)
- C1: Clinical Medicine
- C2: Clinical Surgery
- C3: Other Clinical Investigations

TRAINING/INSTRUCTIONAL (T)
- T1: Training, Education, and/or Instruction for Personnel
- T2: Other Training/Instruction

ADJUNCTS/ALTERNATIVES TO ANIMAL STUDIES (A)
- A1: Adjuncts to Animal Use Research
- A2: Alternatives to Animal Investigation
- A3: Other Alternatives/Adjuncts

CLASSIFIED SECRET OR ABOVE STUDIES (S): Classified secret or above studies on animals

ANIMAL BREEDING STOCK (B): Animals maintained for breeding

OTHER ANIMAL USE CATEGORIES (O): Other animal use purposes

V.1.3 USDA Pain Categories

The USDA requires that all institutions using any regulated animal for research, testing, training, or experimentation register with the USDA as a research facility and submit an annual report. This annual report presents the number of regulated animals used and the type of pain, if any, the animals were exposed to.

The USDA has developed three pain categories for its reporting requirement (TableV-2). All animals herein reported are assigned to one of the three USDA pain categories; this includes animals that are not regulated by the USDA. The USDA requires that any reporting facility that uses procedures producing unalleviated pain or distress file an explanation of the procedures with its annual APHIS report.

The animals reported in Column C of the USDA report are those used in procedures that are not painful. Procedures performed on these animals are those that are usually conducted on humans without anesthesia or analgesia. Examples include most blood-sampling techniques (excluding intracardiac and periorbital blood sampling), injections, and tattooing.

The animals reported in Column D of the USDA report are those that experience pain in which appropriate anesthetic, analgesic, or tranquilizing drugs were used. Examples include anesthesia for surgical procedures or catheter placement, and analgesia during recovery from surgery.

The animals reported in Column E of the USDA report are those that experience more than slight or momentary pain or distress that cannot be alleviated by drugs. Examples of procedures where drugs were not used because they would have adversely affected the procedures, results or interpretation of the research, or tests include some infectious disease studies and some toxicology studies.

All procedures that involve animals in Columns D or E are extensively reviewed during the protocol approval process. A veterinarian with experience and/or training in laboratory animal medicine must review all procedures that could cause pain and distress in animals prior to formal protocol review. In addition, the primary investigator must write a justification for all procedures for animals in

Table V-2 USDA Pain Categories (USDA APHIS Form 7023)

USDA COLUMN C
Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.

USDA COLUMN D
Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.

USDA COLUMN E
Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

Columns D and E. The DoD standard protocol states, "Procedures causing more than transient or slight pain that are unalleviated must be justified on a scientific basis in writing by the primary investigator. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized." Moreover, the primary investigator must sign an assurance statement that alternative procedures are not available, and the Institutional Animal Care and Use Committee must review and approve all procedures before the study begins.

V.2 Results/Discussion

V.2.1 General Results

There was a total of 316,048 animals used in FY97 which is a 1% decrease from FY96 and a 43% decrease from FY93 (Figure V-1). The Animal Welfare Act of 1985 defines animals as "any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal, as the Secretary may determine..." Therefore, only 7% (22,014) of the animals used by the DoD in FY97 are considered USDA reportable species.

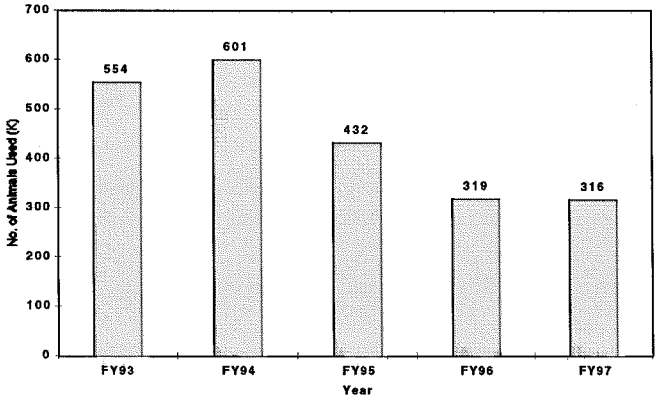


Figure V-1 DoD Animal Use by Year

In FY97, 149,187 animals were used in intramural research programs and 166,861 were used in extramural grants or contracts (Figure V-2). Intramural animal use decreased by 17%, (30,968) in FY97 compared with FY96 use and decreased by 44% (118,904) compared with FY94 use. While the number of animals used in extramural research was 20% (28,216) greater in FY97 than the number in FY96, it was 50% (165,731) lower than the number used in FY94. Extramural programs by their very nature have large

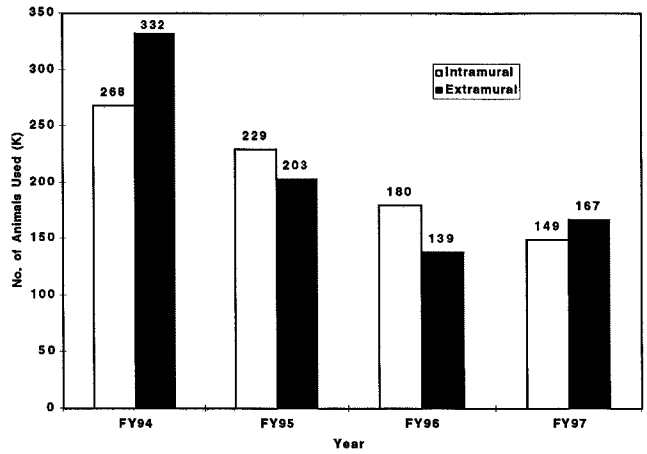


Figure V-2 Intramural/Extramural Animal Use by Year

fluctuations in the number of animals used from year to year. Each year a different number of contracts are granted to perform extramural research. Many of these do not use animals at all; others only use animals during a portion of the proposed project (e.g., third year of project); and others use animals throughout the entire project. In addition, the level of funding for extramural programs varies from year to year thereby changing the total number of extramural projects. Some extramural research programs are congressionally mandated such as Breast Cancer, Neuro-fibromatosis, Osteoporosis Research Programs; their funding is dependent on yearly congressional appropriations. Therefore, changes in the number of animals used by the DoD extramural research programs can fluctuate significantly from year to year. The intramural programs have less variation in their use of animals because they have a continuous mission and ongoing research in specific areas. Consequently, any decrease in the number of animals used is most likely a result of the use of alternatives to animal use, a decrease in the number of research projects, or a decrease in intramural funding.

V.2.2 Animal Use by Service

Information concerning total DoD use of animals by each service is presented in Figure V-3. Figures V-4 and V-5 show the intramural and extramural animal use by service, respectively.

In FY97, the Army used 72% of the total number of animals used by the DoD, 65% of the intramural animals, and 78% of extramural animals. There was

TOTAL = 316,048

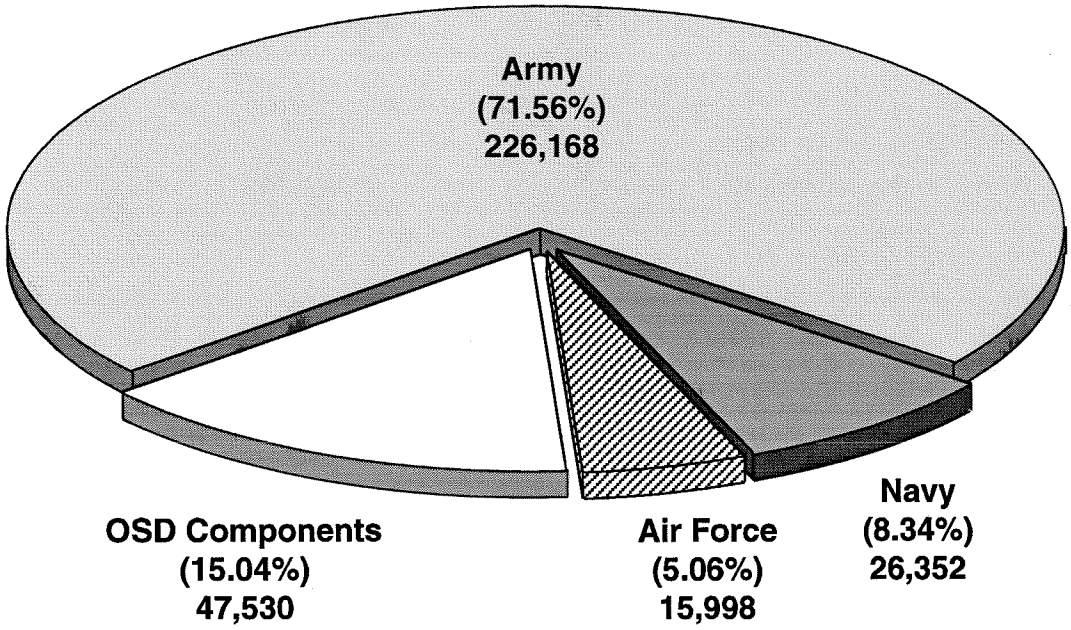


Figure V-3 DoD Intramural and Extramural Animal Use by Service for FY97

TOTAL = 149,187

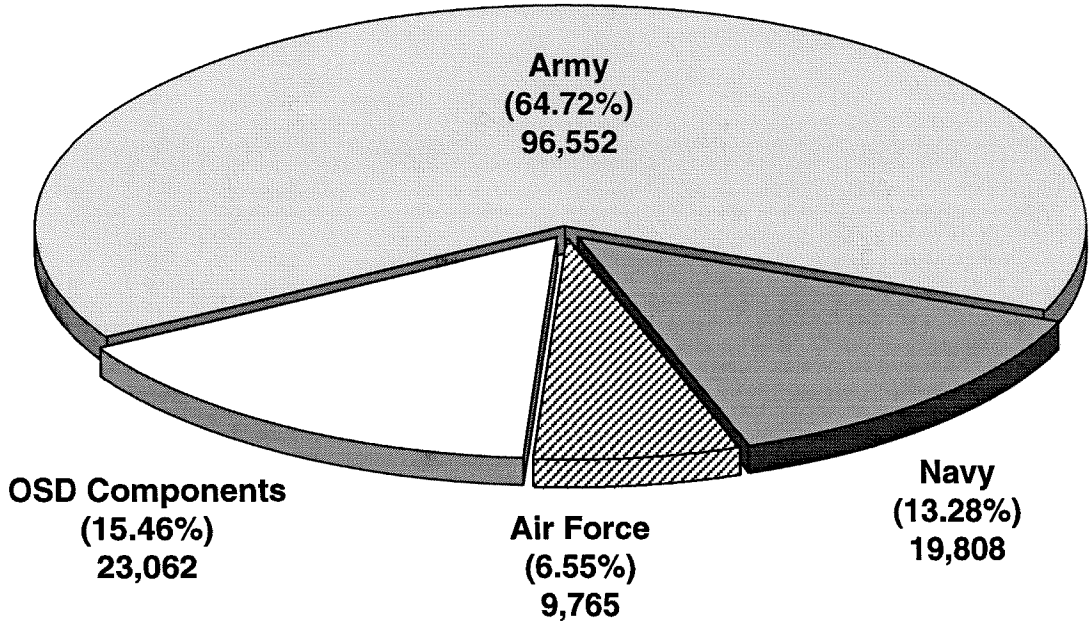


Figure V-4 DoD Intramural Animal Use by Service for FY97

Percentages may not add up to 100% due to rounding of calculations

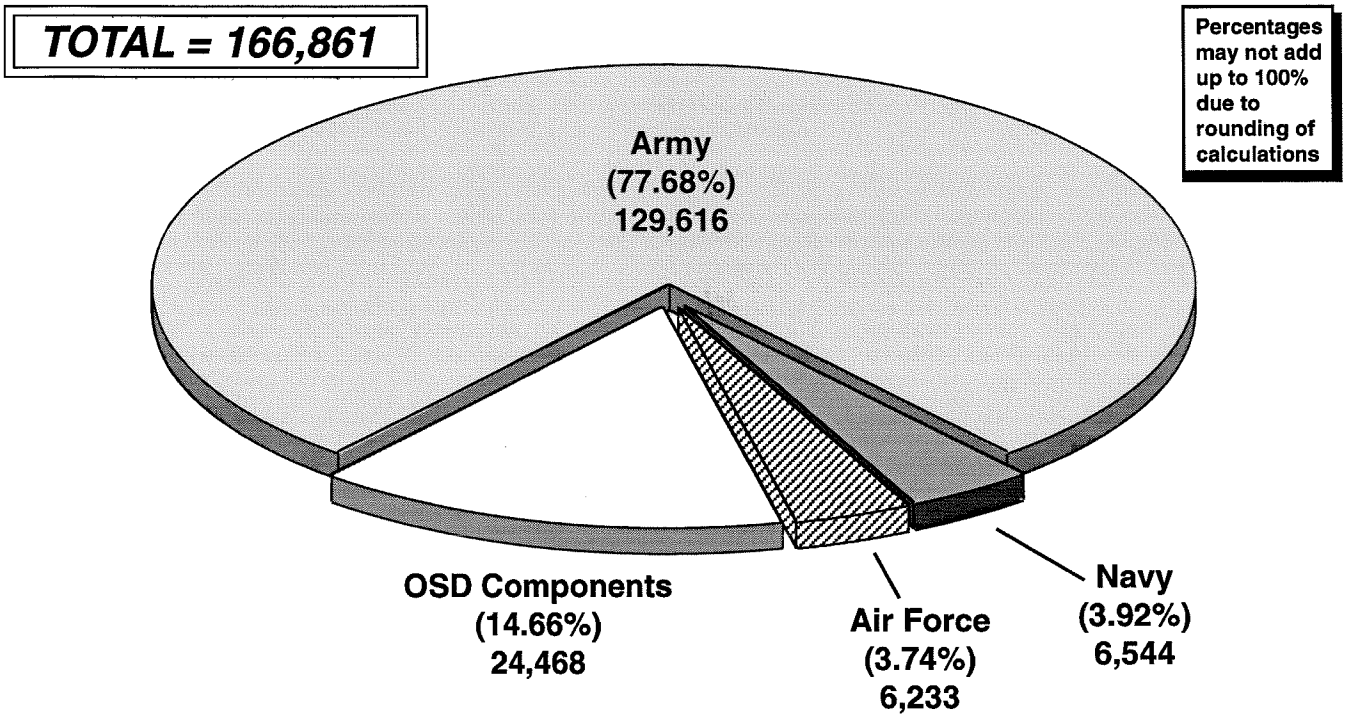


Figure V-5 DoD Extramural Animal Use by Service for FY97

a 9% decrease in the Army's intramural animal use and a 4% increase in extramural animal use since FY96. The Army has an ongoing responsibility to manage the congressionally mandated Breast Cancer, Neurofibromatosis, Osteoporosis, and Defense Women's Health Research Programs. These programs used the majority of the Army's extramural research animals (48,908). The U.S. Army Medical Research and Materiel Command is the congressionally mandated Lead Agency for infectious disease and combat dentistry research and the DoD Executive Agent for medical chemical and biological defense and nutrition studies. The number of animals the Army used in research on infectious diseases and chemical and biological defense was 61,445 and 74,597, respectively.

The Navy used 8% of the total number of animals used by the DoD, 13% of the intramural animals, and 4% of extramural animals. Comparing animal use in FY97 with use in FY96, there was a 29% (10,516) reduction in the total number of animals used by the Navy. Animal use in the Navy's intramural research projects decreased (10,915) while the extramural projects demonstrated a slight increase (399) in animal use. The majority of animals used by the Navy were in infectious disease research (17,014).

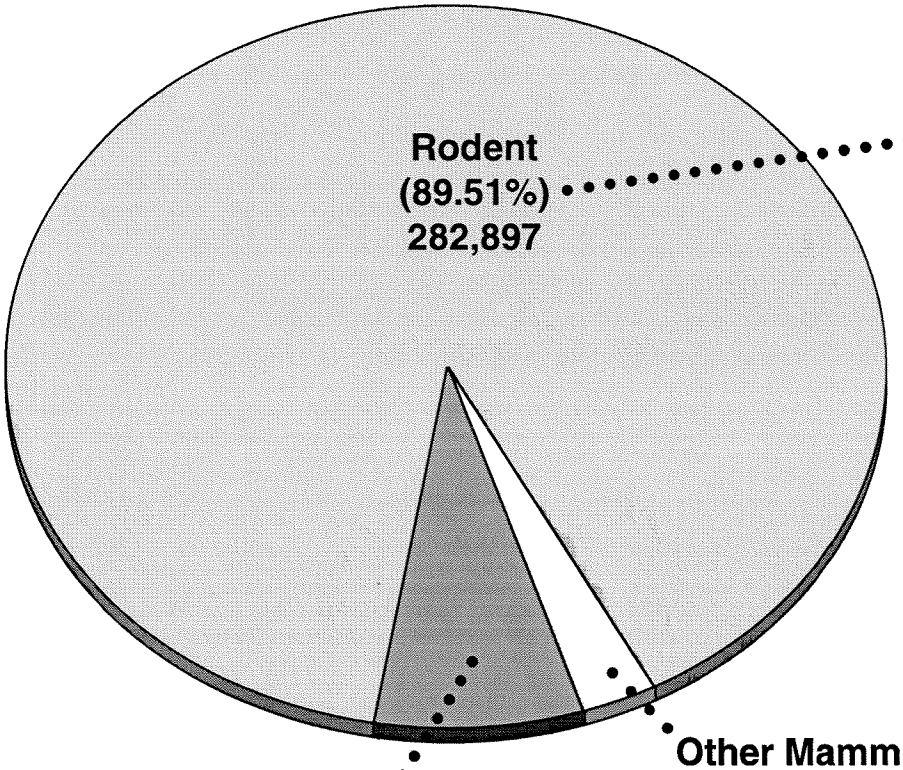
The Air Force used 5% of the total number of animals used by the DoD, 7% of the intramural animals, and 4% of the extramural animals. The Air Force had a slight (2%) increase in intramural animal use and a 16% decrease in extramural animal use resulting in a 6% (995) overall decrease in the number of animals used in research in FY97 compared with FY96. The Air Force used the majority of animals in non-medical research projects (10,789).

The Office of the Secretary of Defense (OSD) components are the Uniformed Services University of the Health Sciences, Defense Advanced Research Projects Agency, Armed Forces Radiobiology Research Institute, and Armed Forces Institute of Pathology. OSD components used 15% of the DoD total animals used, 15% of the total intramural animals, and 15% of total extramural animals. There was a 42% (14,122) increase in the use of animals for the OSD components in FY97 compared with FY96. Over 90% of this increase was in OSD components extramural programs. The OSD components used the majority (96%) of their animals in clinical investigations and medical research.

V.2.3 Animal Use by Species

DoD animal use by species is presented in Figure V-6. Figures V-7 and V-8 represent the intramural and extramural animal use by species for FY97.

TOTAL = 316,048



Chinchilla	193	(0.06%)
Chipmunk	18	(0.01%)
Degu	34	(0.01%)
Gerbil	77	(0.02%)
Guinea Pig	7,602	(2.41%)
Hamster	3,960	(1.25%)
Jird	24	(0.01%)
Mouse	227,303	(71.92%)
Rat	42,272	(13.38%)
Shrew	8	(<0.01%)
Squirrel	36	(0.01%)
Vole	1,370	(0.43%)

Other Mammals
(2.75%)
8,692

Bat	26	(0.01%)
Burro	2	(<0.01%)
Cat	57	(0.02%)
Cow/Bull	8	(<0.01%)
Dog	342	(0.11%)
Ferret	211	(0.07%)
Goat	839	(0.27%)
Horse	4	(<0.01%)
Marine Mammal	303	(0.10%)
Nonhuman Primate	1,527	(0.48%)
Opossum	6	(<0.01%)
Pig/Swine	2,274	(0.72%)
Rabbit	2,709	(0.86%)
Raccoon	14	(<0.01%)
Sheep	370	(0.12%)

Marine Mammals include: Beluga Whale (5), Bottlenose Dolphin (51), California Sea Lion (4), Elephant Seal (20), False Killer Whale (2), Gray Whale (1), Killer Whale (1), Marine Mollusc (168), Northern Elephant Seal (46), Pacific Harbor Seal (1), Pacific White-side Dolphin (3), Risso's Dolphin (1).

Nonhuman Primates include: Aotus Monkey (73), Baboon (24), Monkey (812), Rhesus Monkey (258), Other Nonhuman Primate (360).

Non-Mammal
(7.74%)
24,459

Amphibian	2,517	(0.80%)
Avian	847	(0.27%)
Fish	20,959	(6.63%)
Reptile	136	(0.04%)

Amphibians include: African Clawed Frog (163), Frog (497), Salamander (1,204), Tadpole (212), Toad (441).

Avian include: Chicken (777), Crane (16), Goose (18), Pigeon (36).

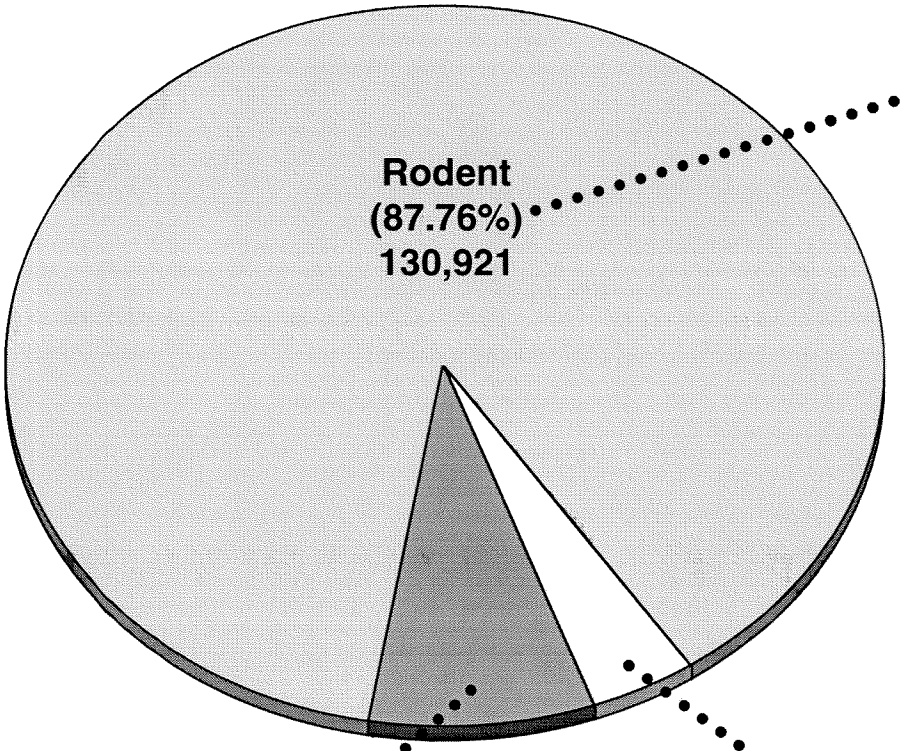
Fish include: Bluegill Sunfish (2,850), Boxfish (7), Eel (27), Fathead Minnow (1,680), Japanese Medaka (10,400), Kniff-fish (9), Milkfish (7), Rainbow Trout (1,000), Triggerfish (27), Zebra Fish (2,000), Other Fish (2,952).

Reptiles include: Iguana (42), Sea Turtle (2), Snake (92).

Percentages may not add up to 100% due to rounding of calculations

Figure V-6 DoD Intramural and Extramural Animal Use by Species for FY97

TOTAL = 149,187



Chinchilla	145	(0.10%)
Chipmunk	18	(0.01%)
Gerbil	42	(0.03%)
Guinea Pig	4,700	(3.15%)
Hamster	2,448	(1.64%)
Jird	24	(0.02%)
Mouse	102,712	(68.85%)
Rat	20,752	(13.91%)
Shrew	8	(0.01%)
Squirrel	7	(<0.01%)
Vole	65	(0.04%)

Non-Mammal
(8.47%)
12,630

Amphibian	1,652	(1.11%)
Avian	213	(0.14%)
Fish	10,710	(7.18%)
Reptile	55	(0.04%)

Amphibians include: African Clawed Frog (35), Frog (200), Salamander (1,204), Tadpole (212), Toad (1).

Avian include: Chicken (143), Crane (16), Goose (18), Pigeon (36).

Fish include: Bluegill Sunfish (2,850), Japanese Medaka (7,000), Other Fish (860).

Reptiles include: Iguana (12), Snake (43).

Other Mammals
(3.78%)
5,636

Burro	2	(<0.01%)
Cat	35	(0.02%)
Cow/Bull	3	(<0.01%)
Dog	157	(0.14%)
Ferret	142	(0.11%)
Goat	818	(0.55%)
Horse	4	(<0.01%)
Marine Mammal	204	(0.14%)
Nonhuman Primate	954	(0.64%)
Opossum	6	(<0.01%)
Pig/Swine	1,536	(1.03%)
Rabbit	1,510	(1.01%)
Raccoon	14	(0.01%)
Sheep	187	(0.13%)

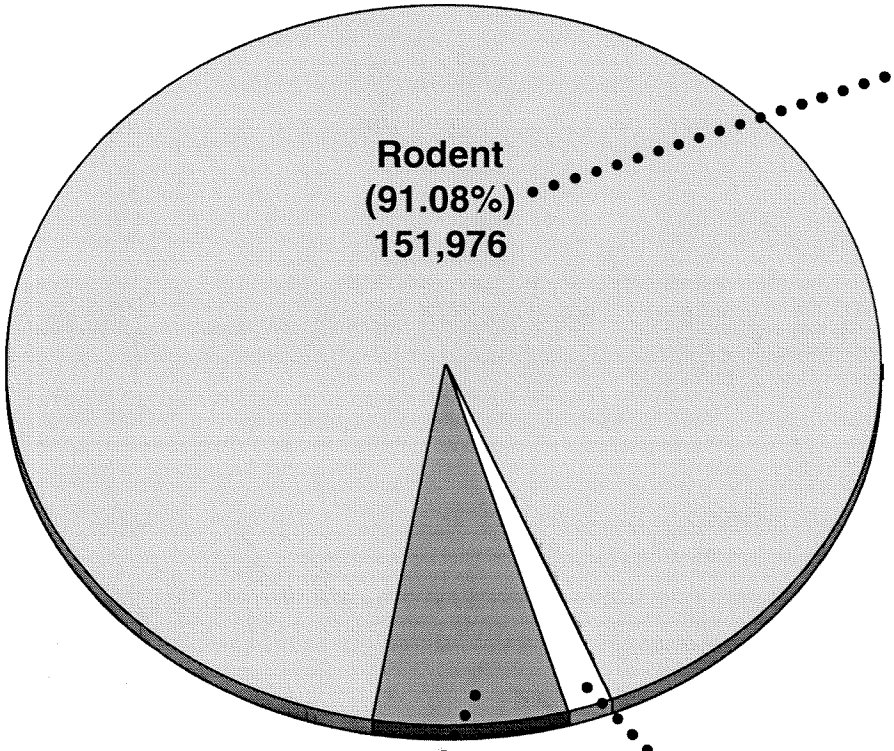
Marine Mammals include: Beluga Whale (3), Bottlenose Dolphin (31), False Killer Whale (1), Marine Mollusc (168), Risso's Dolphin (1).

Nonhuman Primates include: Aotus Monkey (73), Rhesus Monkey (246), Other Monkey (287), Other Nonhuman Primates (348).

Percentages may not add up to 100% due to rounding of calculations

Figure V-7 *DoD Intramural Animal Use by Species for FY97*

TOTAL = 166,861



Chinchilla	48	(0.03%)
Degu	34	(0.02%)
Gerbil	35	(0.02%)
Guinea Pig	2,902	(1.74%)
Hamster	1,512	(0.91%)
Mouse	124,591	(74.67%)
Rat	21,520	(12.90%)
Squirrel	29	(0.02%)
Vole	1,305	(0.78%)

Non-Mammal
(7.09%)
11,829

Amphibian	865	(0.52%)
Avian	634	(0.38%)
Fish	10,249	(6.14%)
Reptile	81	(0.05%)

Amphibians include: African Clawed Frog (128), Frog (297), Toad (440).

Avian include: Chicken (634).

Fish include: Boxfish (7), Eel (27), Fathead Minnow (1,680), Japanese Medaka (3,400), Kniff Fish (9), Milkfish (7), Rainbow Trout (1,000), Triggerfish (27), Zebra Fish (2,000), Other Fish (2,09).

Reptiles include: Iguana (30), Sea Turtle (2), Snake (49).

Other Mammals
(1.83%)
3,056

Bat	26	(0.02%)
Cat	22	(0.01%)
Cow	5	(<0.01%)
Dog	136	(0.08%)
Ferret	54	(0.03%)
Goat	21	(0.01%)
Marine Mammal	99	(0.06%)
Nonhuman Primate	573	(0.34%)
Pig/Swine	738	(0.44%)
Rabbit	1,199	(0.72%)
Sheep	183	(0.11%)

Marine Mammals include: Beluga Whale (2), Bottlenose Dolphin (20), California Sea Lion (4), Elephant Seal (20), False Killer Whale (1), Gray Whale (1), Killer Whale (1), Northern Elephant Seal (46), Pacific Harbor Seal (1), Pacific White-side Dolphin (3).

Nonhuman Primates include: Baboon (24), Monkey (525), Rhesus Monkey (12), Other Nonhuman Primate (12).

Percentages may not add up to 100% due to rounding of calculations

Figure V-8 *DoD Extramural Animal Use by Species for FY97*

The majority (97%) of animals used by the DoD, both intramurally and extramurally, were rodents, birds, amphibians, reptiles, and fish.

The overall number of nonhuman primates, dogs, and cats continued to decrease in FY97 (Figure V-9). Compared with FY96, in FY97 there was a decrease in the use of nonhuman primates (284) and cats (21) and a slight increase in the use of dogs (65). Dogs were primarily used in medical and clinical research (71%).

Since FY94, there has been a 31% (682) decrease in the use of nonhuman primates and a 64% (704) decrease in the use of companion animals for research in the Department of Defense. This illustrates the Department's continuing commitment to reducing the use of specific species in research.

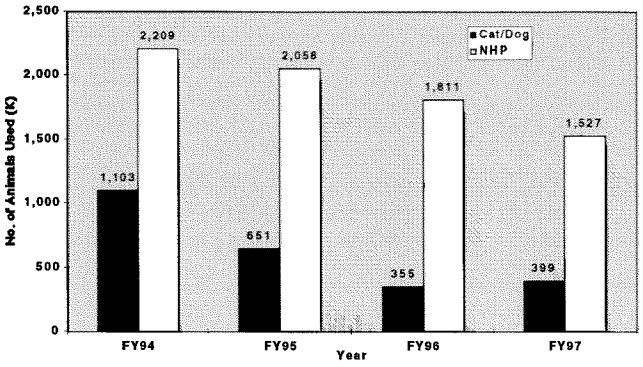
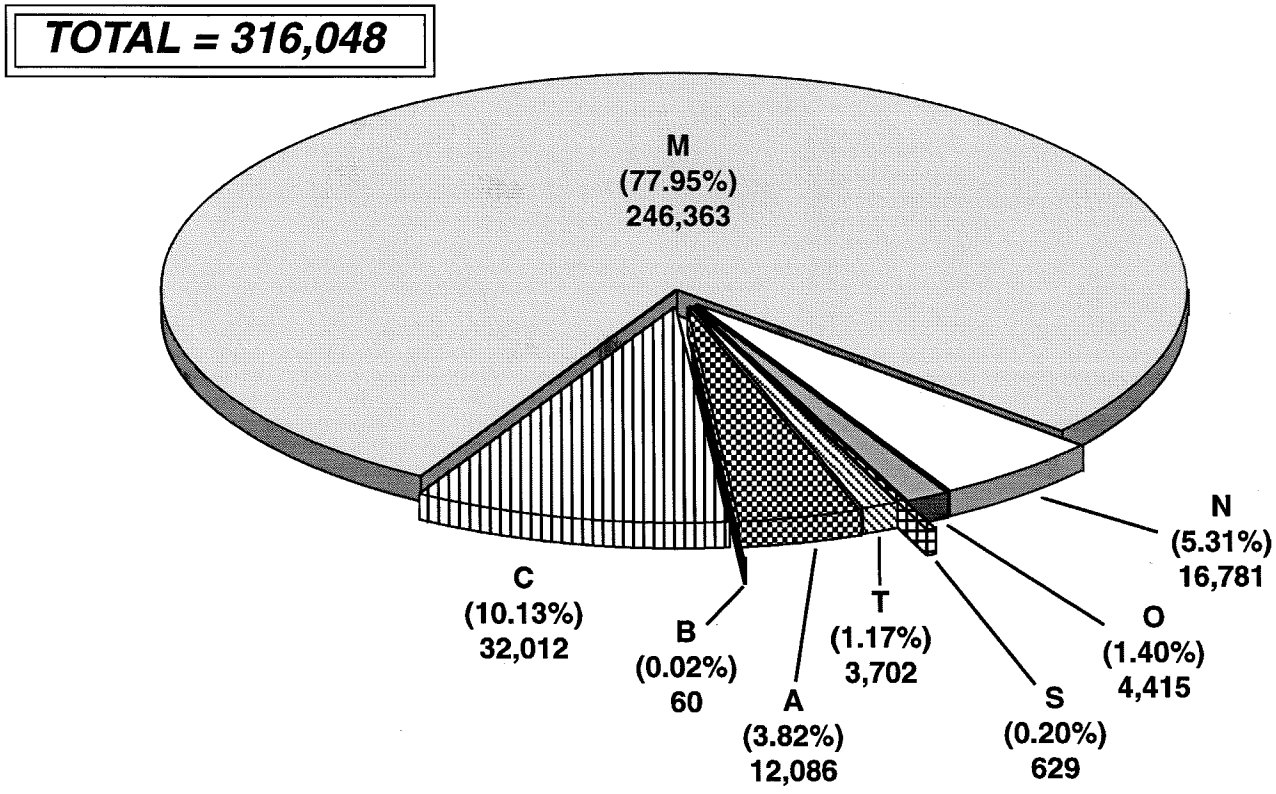


Figure V-9 *Use of Nonhuman Primates, Dogs, and Cats by Year*

V.2.4 Animal Use by Category

Total animal use in the DoD by category is presented in Figure V-10, with the intramural and extramural breakouts in Figures V-11 and V-12, respectively.



A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

Figure V-10 *DoD Intramural and Extramural Animal Use by Category for FY97*

TOTAL = 149,187

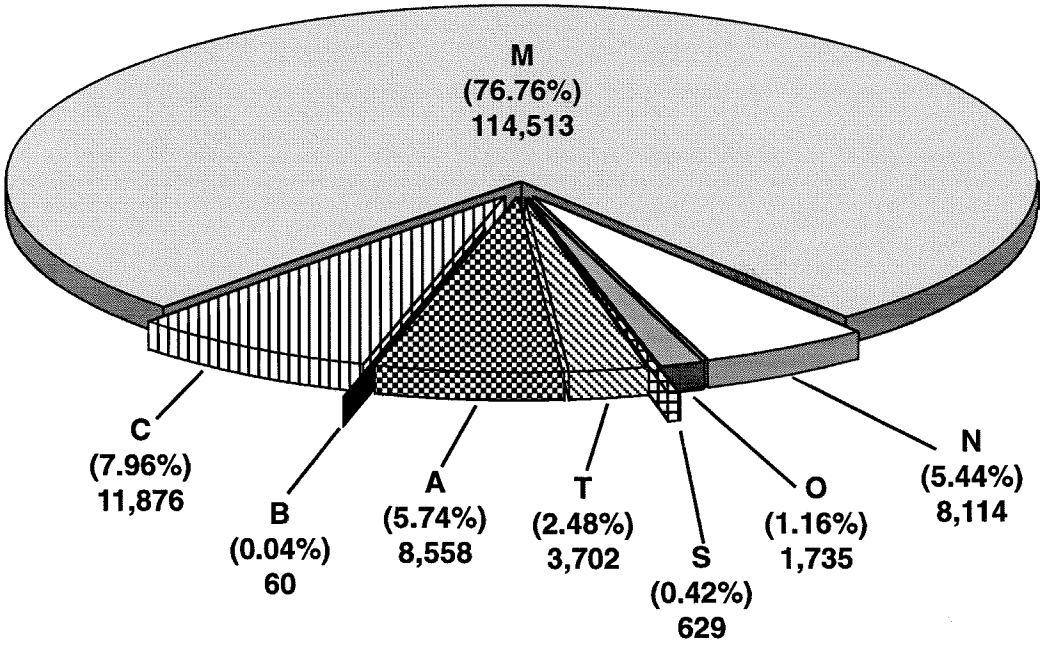


Figure V-11 DoD Intramural Animal Use by Category for FY97

TOTAL = 166,861

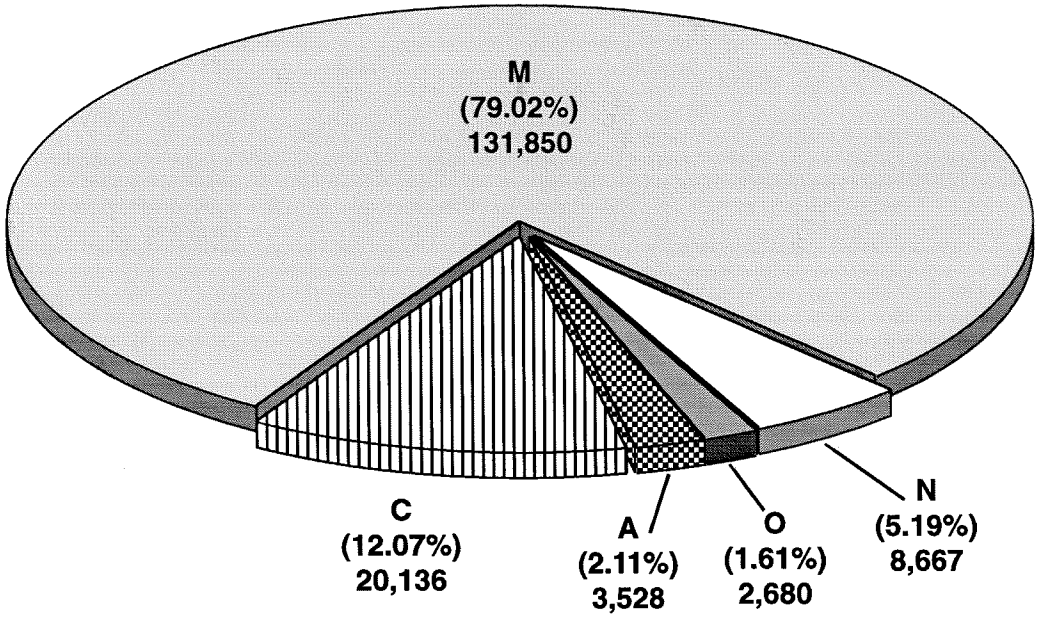


Figure V-12 DoD Extramural Animal Use by Category for FY97

A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

The DoD has a critical and challenging mission: to discover, design, and develop military medical countermeasures against threats to the health and survivability of military personnel. In order to meet this mission, 78% of the animals used by the DoD in FY97 were in medical research. Thirty-five percent (87,043) of animals used in medical research were in the area of infectious diseases (M2) and were primarily rodents (99%) (Appendix O). The primary thrust of this research is the development of preventive measures against infectious disease through discovery, design, and development of prophylactic, therapeutic, and treatment drugs for relevant diseases. The chemical defense research program (M3) used 17% (40,966) and the biological defense research program (M4) used 14% (33,631) of the medical research animals. Medical biological defense develops, demonstrates, and fields new vaccines, drugs, and diagnostic kits for the prevention, treatment, and diagnosis of biological warfare agents. This research program protects the armed forces from the consequences of exposure to biological warfare agents and enhances their survivability. M8 (Other Medical Research) accounted for 24% of the total medical research category (Figure V-13). The congressionally directed research programs in the areas of breast cancer, Defense women's health, neurofibromatosis and osteoporosis used 48,908 animals. These programs accounted for 83% of M8 animals (Table V- 3), 20% of the animals used in medical research, and 16% of the total DoD animals used. These types of research programs can cause fluctuations in the total number of animals used from year to year depending on congressional funding levels and direction. Other areas of research within M8 are shown in Table V-3.

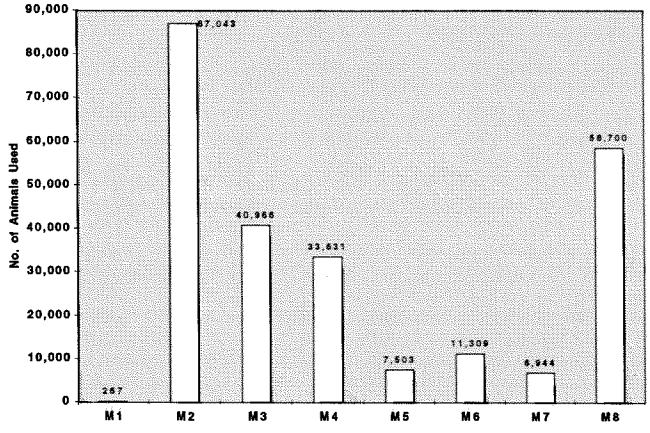


Figure V-13 Animal Use by Medical Research Category

Research Category (M8)	No. of Animals Used	Percentage of M8
Breast Cancer	45,077	76.79%
Diode Lasers	42	0.07%
Defense Women's Health Research Program	1,466	2.50%
Gulf War Illnesses	1,130	1.93%
Medical Free Electron Laser	928	1.58%
Neurobehavioral	288	0.49%
Neurofibromatosis	1,912	3.26%
Occupational Medicine	264	0.45%
Osteoporosis	453	0.77%
Pathophysiology	1,406	2.40%
Toxicology	2,339	3.98%
Vectorborne Disease Research and Training	6	0.01%
Zoonosis	3,389	5.77%
Total M8 Research	**58,700**	**100.00%**

***Table V-3** M8 (Other) Medical Research Category*

Clinical research accounted for 10% (32,012) of the animals used by the DoD in FY97. Studies in this category address clinical medicine and surgical problems for the treatment of both diseases and combat casualties. Ninety-two percent of the animals used in clinical research were used in clinical medicine studies. While many of these conditions are unique to the military, several are not. Specific types of clinical studies are listed in Appendix N.

One percent of the animals used by the DoD in FY97 were in the training, education, and instruction of personnel. Training and instruction are basically for animal technicians and medical personnel (Appendix N). There was a 45% decrease (2,978) in animals used in this category in FY97 compared with FY96. The reduction in the use of animals for training purposes reflects the DoD's commitment to using non-animal training techniques such as computer simulations and sharing of training animals whenever possible. Breeding stock, classified studies, and other studies accounted for less than 1% of the DoD's total animal use in FY97.

Non-medical RDT&E animal use decreased by 59% (24,164) in FY97 compared with FY96 and accounted for only 5% of the total animal use in FY97. Research in the area of alternatives to the use of animals was 4% of the total animal use for FY97. Research in this category illustrates the Department's continuing initiatives to promote research to develop alternatives to reduce, replace, and refine the use of animals in DoD research.

No animals were used for offensive weapons testing during FY97.

V.2.5 Animal Use by USDA Pain Category

Total animal use in the DoD by USDA pain category is presented in Figure V-14, with the intramural and extramural breakouts in Figures V-15 and V-16, respectively.

Most research (~80%) in the DoD was not painful to the animals involved. In the majority of the cases (52%), the animals were not exposed to or involved in any painful procedures. In 28% of the cases, animals were given anesthesia or pain-relieving drugs during procedures that could have involved some pain or distress to the animals. In 20% of the animals used, anesthetics or analgesics were not used because they would have interfered with the validity of the results of experiments. Most (98%) of the animals used in painful experiments (where reducing the pain or distress would have interfered with the validity of the results) were rodents. Less than 1% of the animals in USDA Pain Category E were other mammals and less than 1% were non-mammals.

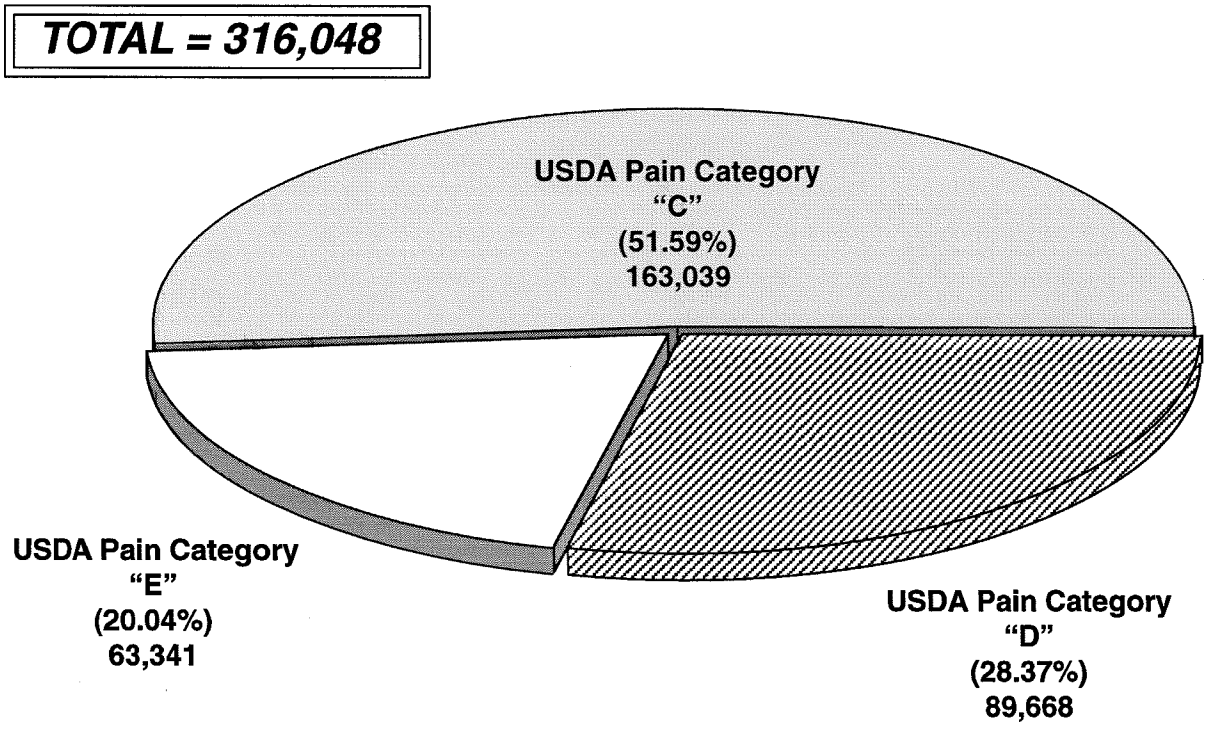


Figure V-14 DoD Intramural and Extramural Animal Use by USDA Pain Category for FY97

TOTAL = 149,187

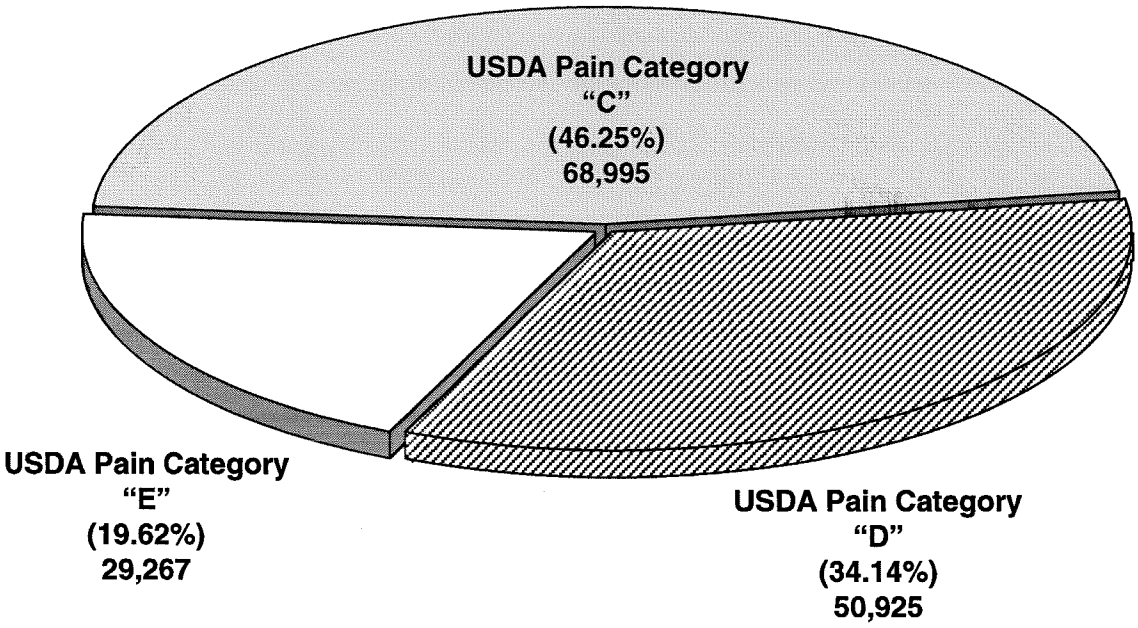


Figure V-15 DoD Intramural Animal Use by USDA Pain Category for FY97

Percentages may not add up to 100% due to rounding of calculations

TOTAL = 166,861

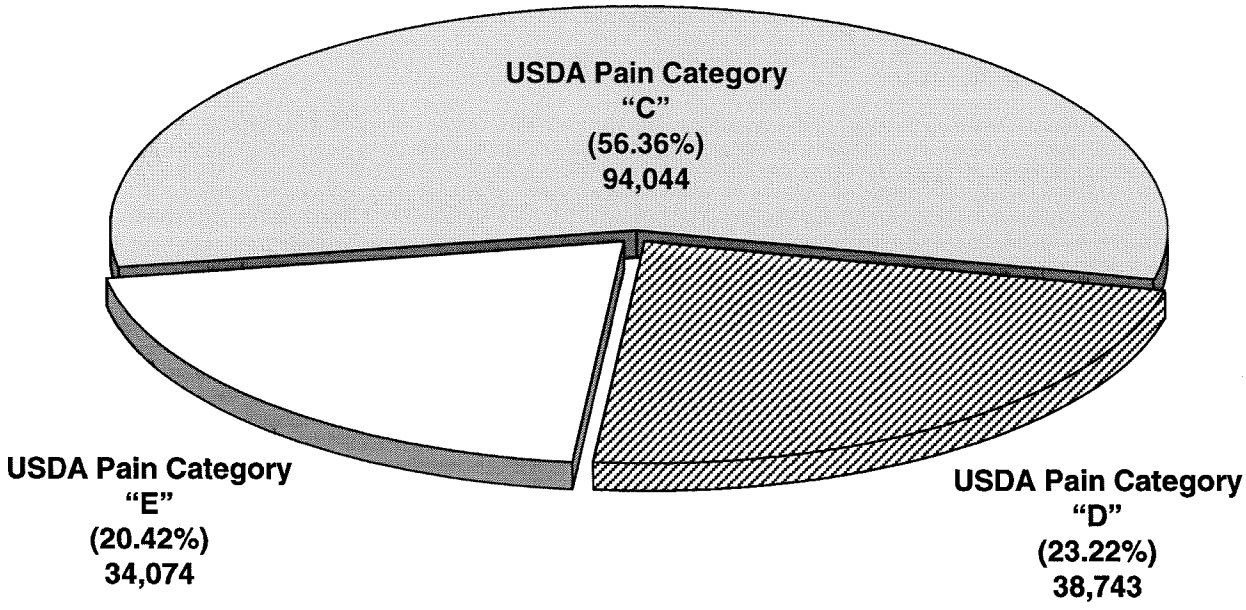


Figure V-16 DoD Extramural Animal Use by USDA Pain Category for FY97

Percentages may not add up to 100% due to rounding of calculations

Ninety-four percent of the animals reported in USDA Pain Category E were used in medical studies; of this, 87% of the animals were used in research on infectious disease and chemical and biological defense. Infectious disease and chemical and biological defense research usually falls into USDA Pain Category E because the animals have to be exposed to chemical or biological agents or other infections which may result in some type of distress. There were no animals subjected to unalleviated pain during training studies.

The DoD clearly has a most diverse, unique, and demanding R&D mission. The modern battlefield is a hostile and dangerous environment with extraordinary potential for exposure to lethal or debilitating conventional weapons, exotic endemic diseases, biological and chemical agents, nuclear blast and radiation, directed energy sources, and complex and dangerous equipment. In addition, a host of adverse environmental conditions, such as cold, heat, high and low pressure, and G-forces are threats to service men and women. The DoD must provide acceptable protection against these threats and many others. The animals reported in USDA Category E were used in research designed to find ways to protect service men and women from the threats they encounter daily. Note that in most of these studies the distress level is minor such as in heat stress or gastrointestinal distress after being exposed to G-forces. This critical research is often reliant upon animal models for vaccine and efficacious countermeasure development. Research of this kind is not commonly done elsewhere in the government, academic, or private sectors and therefore is the sole purview of the DoD. Also, a large portion of these studies are driven by federal requirements, particularly those of the Food and Drug Administration.

SECTION VI

DoD INITIATIVES TO PROMOTE ALTERNATIVE METHODS THAT REPLACE, REDUCE AND REFINE THE USE OF ANIMALS

Alternatives, as articulated in *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), are defined as methods that Replace, Reduce and Refine the use of animals. In addition to these *Three Rs*, the Department of Defense (DoD) advocates a fourth *R*, "Responsibility," for implementing these alternative methods.

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."

Reduction

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

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.

Responsibility

The DoD has taken responsibility for implementing animal use alternatives. This commitment illustrates the DoD's initiative toward utilization and development of alternatives to animal use.

Department policy with regard to animal alternatives is promulgated in DoD Directive 3216.1 which directs that "it is DoD policy that... alternatives to animal species should be used if they produce scientifically satisfactory results...." This policy is implemented in the Joint Service Regulation on the Use of Animals in DoD Programs, which delegates responsibility to the local commander for utilization of alternatives to animals.

To illustrate the Department's initiatives to promote these *Four Rs*, a description of such initiatives within DoD's research laboratories and medical treatment centers is provided. The following list is not all inclusive, as the number of specific examples of implementing alternative methods that can be documented for DoD's research projects is large. Rather, it illustrates the scope, diversity, and spirit of DoD's *Four Rs* initiatives. This section will demonstrate a broad-based movement toward the use of biotechnology and other innovative adjuncts to replace and reduce animal use as well as refinement in methods used in essential animal studies.

VI.1 DoD DEVELOPMENT OF ANIMAL USE ALTERNATIVES

A review of the FY97 DoD research reveals that 13 DoD facilities were actively involved in the development of alternatives to animal use, which is a 40% increase from FY96. These developments occur through both research specifically designed to produce alternatives and by research to improve experimental techniques. Whenever possible, DoD investigators attempt to develop state-of-the-art, scientifically relevant and reliable experimental procedures that can be performed without the use of animals. In addition, in cases where the animal models cannot be completely replaced, investigators work diligently to develop refinement techniques to reduce any stress placed on the animal during both experimental procedures and daily living. Examples of alternatives developed or in

development by DoD investigators reported in 1997 are listed below. This is only a sample of the alternatives developed or being developed this year.

Replacement:

- Rabbits replace nonhuman primates (NHPs) in the development of an *in vitro* correlate for anthrax.
- The South African clawed frog (*Xenopus laevis*) embryo replaces laboratory mammals commonly used in teratogenesis assays.
- Use of cell culture assays for determining the neutralization titers of mouse sera in Alphavirus vaccine research replaces the requirement for additional animals.
- Development of polymerase chain reaction techniques to detect *Coxiella burnetii* in clinical specimens will eliminate the need to detect the microorganism using experimental animals.
- The Japanese medaka fish used as an immune response biomarker should replace laboratory animals commonly used in toxicology research.
- Realistic biophysical models computationally simulate the damage processes induced by lasers as accurately as possible.
- Using the zebrafish as a model organism will replace higher order mammalian species for genetic analysis of vertebrate circadian rhythmicity.
- Artificial human skin and human whole blood can be used to delineate mechanisms and sites of action for stimulants and potential vesicant antagonists.
- Rabbit models will replace the use of dogs or NHPs in the study of compartment pressures in ischemia reperfusion.
- The free-ranging honeybee (*Apis mellifera*) is being used in place of animal sentinels to monitor contaminated environments.
- A technique was developed to examine individual neurons in culture, eliminating the need for intracranial surgery.
- Anatomical computer models of experimental animals and computer codes calculate where and how much microwave energy is absorbed in experimental animals when exposed to radiofrequency fields.
- An artificial eye with lenses that mimics the focusing characteristics of the eye can be used to expose this eye to various thresholds of laser exposures.

Reduction:

- The guinea pig model of anthrax vaccine adsorbed against strains of *Bacillus anthracis* from different, worldwide geographical areas will reduce the number of rabbits and NHPs that would be used to investigate *Bacillus anthracis* strain difference.
- A computer-based dosimetry model on the effects of microwave radiation on cognitive performance will reduce the numbers of NHPs needed in future experiments.
- Development of a rabbit model for passive immunization studies against *Bacillus anthracis* results in a reduction in the number of NHPs used for future studies.
- Using cell culture assay for initial determination of antiviral activity greatly reduces (>90%) the number of compounds requiring testing in animals.
- Isolated perfused porcine skin flap technique generates more precise data, thus reducing the number of experimental replicates (animals) needed to obtain data on the dermal absorption of chemicals.
- Combining teaching labs and other labs that use the same animals decreases the number of animals used by 50%.
- A technique to record *in vivo* electrophysiological activity of brain neurons by implanted telemeters allows more data to be obtained from one animal over long time periods instead of using many animals for short periods.

- Development of non-animal systems such as cell culture for virus titrations and plaque-reduction neutralization tests in Encephalomyelitis virus (VEE, EEE, and WEE) challenge studies has substantially reduced the use of animals for these determinations.

- Cell culture studies will be used to characterize infectivity of preparations before animals are used thereby reducing the number of animals needed.

Refinement:

- Biomechanical comparison of electrosurgical and scalpel incisions has been refined by the use of state-of-the-art surgical equipment and technique combined with long-acting post-op analgesics and specially designed recovery area.

- Development of a preparation of viral antigens in a rabbit kidney cell line with rabbit serum or in serum-free culture should result in less reaction by the animals.

- Propagating the Leishmania organism in the tail of the jird greatly reduces the apparent distress experienced by the subject.

- Development of a training program on the primate equilibrium platform will make it possible to demonstrate subtle toxic effects on performance at doses that do not produce frank toxicity or anything approaching life-threatening condition.

VI.2 DoD Implementation of Animal Use Alternatives

DoD research protocols strive to minimize the number of animals used to accomplish the program mission and goals. During the review of protocols by the Institutional Animal Care and Use Committee (IACUC), investigators are specifically asked to present information indicating that "Reduction, Refinement, and Replacement" have been addressed in the animal study. Implementation of these alternatives reduces, replaces and refines the Department's use of animals in research. This is accomplished by the implementation of both general and specific alternatives. General alternatives are those that are frequently implemented in many different DoD programs. Specific alternatives are those that may be specific to both a research protocol and/or facility. In FY97, over 400 animal use projects reported that they were implementing alternative methods to the use of animals. Since over 600 general and specific alternatives were implemented by the DoD this year, it is impossible to present all of them in this report. The following examples are a representative listing of general alternative methodologies commonly practiced in DoD facilities:

Replacement:

- During the review process, all potential methods of adequately answering the research objective are reviewed prior to the use of an animal model.

- The evaluation process also considers the selection of a particular animal type; species lower on the phylogenetic scale are considered and used if its selection permits attainment of the research objectives.

- Non-animal training aids are used to reduce the number of live animals required.

Reduction:

- All animal use protocols are subject to review by a biostatistician who addresses the animal used, study design, and statistical evaluation packages, and ensures that the minimum number of animals will be used to meet the specific scientific objectives.

- Pilot studies are used to refine techniques and define the animal model so that animal use can be kept to the minimum required for statistical significance.

- When possible, protocols make use of a repeated measures design and each animal serves as its own control, thereby reducing the number of animals necessary for a particular study.

- Sharing of animal tissues with other investigators reduces animal use.

- Collaboration between DoD investigators allows for a single animal to be used in multiple training and research procedures and the sharing of control group information resulting in an overall reduction in the number of animals used.

- Several types of data are collected simultaneously.

- Training sessions are designed to use the highest student-to-animal ratio that is practical.

Refinement:

- Parameters indicating moribundity rather than death are used as experimental endpoints when possible.

- Animals are anesthetized during euthanasia to decrease stress. Tissues will be maximally utilized to decrease the number of animals needed to supply tissues for this experiment.

- Moribund animals are humanely euthanized to prevent unnecessary pain or distress.

- Utilizing the environmental enrichment strategy, animals are housed in social settings (i.e., pairs or groups) in an enriched environment (e.g., nestboxes, toys).

Specific alternatives implemented by the DoD in FY97 were categorized as a subset of replacement, reduction or refinement and are shown in Table VI-1. These categories illustrate the broad-based spectrum of alternatives to be implemented by the DoD. A representative listing of the specific alternatives is presented in Appendix P.

In addition to the implementation of alternatives, the DoD has established policies specific to the refinement of animal use. For example, Walter Reed Army Institute of Research (WRAIR) has established a policy that mandates consideration of environmental enrichment for research animals. This policy allows for flexibility and creativity for improving conditions of laboratory animals.

***Table VI-1** Alternatives Categories*

Replacement:
- Non-mammalian species or species lower in the phylogenetic scale
- Biochemical/physical methods
- Computer simulations
- Other species replace companion animals
- Replacement using *in vitro* cell cultures

Reduction:
- Utilization of alternative biological testing method
- Substitution of computer simulations or other technologies
- Substitution of another species
- Changes in endpoint measurements

Refinement:
- Reduce pain
- Reduce distress
- Research models and animal alternatives
- Environmental enrichment and improved animal handling

VI.3 DoD Initiatives to Promote Animal Alternatives

The DoD has established a variety of initiatives and targeted programs that are currently in place to promote alternative methods that will refine, reduce and replace the use of animals. These programs are designed to target individual and institutional awareness by providing educational opportunities, professional training, and fiscal resources toward implementing the *Four Rs* approach to animal use.

VI.3.1 Science and Technology Objectives to Reduce Reliance on Animal Research

The Department of Defense continues to seek alternatives to animal use through an Army Science and Technology Plan (STEP) initiated in FY93 and continuing through FY04 titled *Reducing Reliance on Human and Animal Subjects of Research and Improving Experimental Conditions Using Animals*. The objectives of the STEP is to conduct basic research to develop new technologies to incrementally reduce future reliance on research animals. The U.S. Army Medical Research and Materiel Command

(USAMRMC) Medical Biological Defense Research Program budgeted approximately $558,000 in FY97 for this objective, which is available to support alternatives to animal use research.

VI.3.2 DoD-Sponsored Conferences and Workshops on Alternatives to Animal Use

The DoD promotes responsibility for alternatives to animal use by sponsoring major meetings and conferences on the subject. Every 2 years the DoD sponsors an international meeting at Aberdeen Proving Ground on Alternatives to Animal Testing (Table VI-2).

The 1992 meeting had 35 scientific platform sessions and 22 scientific poster presentations. This international symposium was attended by nearly 300 military and civilian scientists from four countries. Proceedings of the 1992 symposium are available through the Defense Technical Information Center (DTIC). In addition, in 1994 a book edited by Dr. Harry Salem titled "Animal Test Alternatives" was published by Marcel Dekker, Inc., which included chapters prepared by most of the presenters at this symposium. The 1994 meeting had 26 scientific platform sessions, including one by Dr. Martin Stephens of the Humane Society of the United States, and 45 scientific poster presentations. This meeting was attended by over 330 military and civilian scientists from seven countries. The proceedings and a monograph based on this successful symposium are available through DTIC. The book "Advances in Animal Alternatives for Safety and Efficacy Testing" has been published by Taylor and Francis. The 1996 conference was coordinated with the Scientists Center for Animal Welfare which held its meeting 10-11 June 1996 to present Animal Welfare and Toxicology/Safety Studies: Current Issues and Trends for the Next Century. The DoD will sponsor another symposium on alternatives to animal use in 1998.

Table VI-2 *DoD-Sponsored Alternatives*

Date	Title
1990	DoD Initiatives in Alternatives to Animal Testing
1992	Current Concepts and Approaches on Animal Test Alternatives
24-26 May 1994	Alternatives in the Assessment of Toxicity: Theory and Practice
12-14 June 1996	Biennial International Symposium on Alternatives in the Assessment of Toxicity Issues, Progress and Opportunities

VI.3.3 National Research Council, Institute of Laboratory Animal Research, Educational Programs

The DoD's priority and continuing commitment to promoting individual and institutional responsibility for alternatives to animal use are reflected in continuing financial support of the Institute of Laboratory Animal Research (ILAR) educational program of the National Research Council. The principal thrust of the ILAR grant is development of institutional training materials, educational courses, and publications in support of the Department's laboratory animal care and use programs. This ILAR information is used in various military research facilities as an important adjunct to existing investigator training and technical education programs on animal care and use. The ILAR information and programs have generated strong animal alternative provisions for both civilian and military-specific research opportunities. The Department has funded this work since 1987 through 5-year grants, and is currently providing funding under the third such grant. In the face of diminishing research funds, the Department has resolved to maintain this important collaboration by providing in excess of $100,000 annually for the ILAR program.

VI.3.4 DoD's Participation in Other Federal Alternatives Programs

The DoD is also represented on the Interagency Regulatory Alternatives Group (IRAG), which planned and presented a "Workshop on Updating Eye Irritation Test Methods" in 1991 and held another workshop on dermal testing held at the American College of Toxicology, in November 1995. The DoD representative on the IRAG (Dr. Harry

Salem) received the Food and Drug Administration's (FDA's) Group Recognition Award for his outstanding contributions to the IRAG (Appendix Q).

The National Institutes of Health Revitalization Act of 1993 (Public Law No. 103-43, Section 1301) directed the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH) to establish an Applied Toxicological Research and Testing Program which represents the NIEHS' component of the National Toxicology Program. The Act further directed the NIEHS to "(a) establish criteria for the validation and regulatory acceptance of alternative testing methods, and (b) recommend a process through which scientifically validated alternative methods can be accepted for regulatory use." To fulfill this mandate, an ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICC-VAM) (the Committee) was established in 1994 by NIEHS to develop a report recommending criteria and processes for validation and regulatory acceptance of toxicological testing methods that would be useful to Federal agencies and the scientific community. The Department of Defense participated in this effort that resulted in a report on the validation and regulatory acceptance of toxicological test methods.

Presentations have also been made on alternatives to the Board of Scientific Councilors of the National Toxicology Program of the National Institute of Environmental Health Sciences (NTP-NIEHS), Board of Scientific Councilors of the Food and Drug Administration and Cancer Etiology Group at the National Cancer Institute.

VI.3.5 Institutional Animal Care and Use Committee Emphasis

Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1-4 of the Code of Federal Regulations has specific provisions for addressing the issue of alternatives during the research animal protocol review process. The DoD has been a leader in forming lawfully constituted and functioning IACUCs at its biomedical research facilities. Accordingly, DoD IACUCs consider alternatives to the proposed use of animals as an important review consideration. All DoD programs use a Standardized IACUC Protocol Format for animal use proposals, which requires that non-animal alternatives be considered. It states that "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." Investigators must provide information on the animal model being proposed and justification for the selected species. The Standard Protocol Format states that "investigators should use the least sentient species that will permit the attainment of research objectives." In addition, the investigators are required to provide a short description of the features of the proposal that may qualify the study as one that refines, reduces or replaces the use of animals. The DoD 1995 Policy Letter requires that extramural contractor proposals utilizing animals in research, testing or training include all the information contained in the DoD Standard Protocol Format, thereby requiring them to also provide the alternatives information.

VI.3.6 Veterinary Staff Expertise and Assistance Visits

The major biomedical research commands of the Military Departments each have credentialed laboratory animal medicine (LAM) veterinarians serving in key staff positions. More than 30 board-certified specialists of the American College of Laboratory Animal Medicine (ACLAM) currently serve in the DoD. In addition to being advisors to commanders on issues related to animal welfare and alternatives to animal use, these veterinarians provide oversight and structure to the command's animal care and use programs. These officers also make periodic staff assistance visits to subordinate facilities that use animals and evaluate each laboratory animal care and use program. Consideration of the use of alternatives is reviewed on these staff assistance visits. Another important responsibility of the LAM veterinarian is to review extramural animal use protocols, ensuring that alternatives to animal use and personnel training issues have been addressed.

VI.3.7 Professional Veterinary Training in LAM

The individuals who are specialty trained in veterinary LAM provide expertise in DoD biomedical research institutions, which strongly

correlates to effective animal use alternatives programs. This is especially true in the critical area of refinements. The DoD has long been a leader in training veterinarians in the field of LAM, the biomedical and veterinary specialty most closely associated with laboratory animal welfare and laboratory animal care and use programs. Many of the nationally prominent leaders of several laboratory animal associations were formally trained in, or closely associated with, DoD LAM training programs. Examples are the President and several past presidents of ACLAM, the President-elect and several past presidents of the American Association of Laboratory Animal Science (AALAS), and several past presidents and the current Secretary-Treasurer of the American Society of Laboratory Animal Practitioners. This traditional DoD strength in LAM expertise strongly enhances both animal care and use and animal alternatives programs. Greater than 25% of all ACLAM boarded specialists in the U.S. received some or all of their LAM training in DoD LAM training programs.

VI.3.8 AALAS Technician and Laboratory Animal Science Training

There are a number of DoD research facilities that sponsor formal training programs leading to certification of animal care and research personnel as AALAS laboratory animal technicians. This specialized training is offered to both government and non-government animal technicians. It is an important mechanism for ensuring highly qualified animal care and research technicians in Defense laboratories. Individual DoD institutions have sponsored formal seminars for research personnel where experts from the National Agricultural Workshop curriculum include formal training and information on alternatives to animal use. In addition, WRAIR offers quarterly a workshop on ethical and administrative issues relating to animal use. The AALAS technicians' course curriculum and the WRAIR workshop curriculum include formal training and information on alternatives to animal use.

VI.4 Summary

Each year new techniques and capabilities improve the handling, treatment, and use of animals in research and testing, and potentially reduce the need for animals in those same endeavors. In FY97, there was ample evidence of the DoD's aggressive pursuit to develop alternatives to replace, reduce, and refine the use of animals, for example, the developed alternatives highlighted in Section VI.1 and the increase in the number of facilities reporting developing animal use alternatives. In addition to these developmental efforts, animal use data for FY97 indicate the widespread implementation of validated alternatives. Rats and mice continue to replace nonhuman primates and other mammals higher on the phylogenetic scale in vaccine and drug development efforts. These and other examples of the development and implementation of alternatives have translated into reductions in the overall use of animals higher on the phylogenetic scale (see Section V). Animal use alternatives including refinement, reduction, and replacement constitute key initiatives in the biomedical research, testing, education, and training programs of the Department of Defense. The number of large animals used by the military departments over the past decade has been significantly reduced, and some large species are rarely used at all. Dogs, cats, nonhuman primates, and marine mammals collectively represent less than 0.7% of the total animals used in research by the DoD.

SECTION VII
GLOSSARY

Adjuvant: An agent mixed in a vaccine to enhance the immunological protection afforded.

Alternatives to Animal Use: For purposes of this assessment, "alternatives" are defined as encompassing any subjects, protocols, or technologies that replace the use of laboratory animals altogether; reduce the number of animals required; or refine existing procedures or techniques so as to minimize the level of stress endured by the animal. These technologies involve the continued, but modified, use of animals; use of living systems; use of chemical and physical systems; and use of computers.

Analgesic: An agent that relieves pain without causing loss of consciousness.

Anesthetic: An agent that causes loss of the sensation of pain. Anesthetics may be classified as topical, local, or general.

Animal: For purposes of this assessment excluding embryos, animal is defined as any nonhuman member of five classes of vertebrates: mammals, birds, reptiles, amphibians, and fish. Within this group, two kinds of animals can be distinguished, warm-blooded animals (mammals and birds) and cold-blooded animals (reptiles, amphibians, and fish). Under this definition, invertebrates are not included.

Animal Use: The use of animals for research purposes. Three aspects of animal use are addressed in this assessment: behavioral and biomedical research; testing products for toxicity; and education of students at all levels. This assessment does not cover animal use for food and fiber; animal use to obtain biological products; or animal use for sport, entertainment, or companionship.

Animal Welfare Act: This act, passed in 1966 and amended in 1970, 1976, and 1985, was originally an endeavor to stop traffic in stolen animals that were being shipped across state lines and sold to research laboratories. Amendments to the act have expanded its scope to include housing, feeding, transportation, and other aspects of animal care; however, the act bars regulation of the conduct of research and testing by USDA.

Antibody: Proactive proteins produced by lymphocytes (type of white blood cell) that can specifically bind foreign substances.

Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC): A voluntary private organization that has provided accreditation for over 600 institutions. AAALAC accreditation is based on the provisions of the NRC *Guide for the Care and Use of Laboratory Animals*, and is recognized by the Public Health Service.

Biological Model: A surrogate or substitute for a process or organ of interest to an investigator. Animals or alternatives can serve as biological models.

Biomedical Research: A branch of research devoted to the understanding of life processes and the application of this knowledge to serve humans and animals. A major user of animals, biomedical research affects human health and the health care industry. It is instrumental in the development of medical products such as drugs and medical devices, and in the development of services such as surgical and diagnostic techniques. Biomedical research covers a broad spectrum of disciplines, such as anatomy, biochemistry, biology, endocrinology, genetics, immunology, nutrition, oncology, and toxicology.

Blast Overpressure: The concussion that results when weapons such as artillery pieces are fired. Soldiers firing these weapons can be severely injured by the local pressure effects resulting from weapon use. Blast overpressure occurs when soldiers are fired upon also, i.e., the shock wave from enemy weapon fire/blast.

Carcinogen: An agent or process that significantly increases the incidence of abnormal, invasive, or uncontrolled cell growth in a population. Carcinogens fall into three classes: chemicals, viruses, and ionizing radiation. A variety of screening assays have been developed to detect chemical carcinogens, including the *Salmonella*-mediated mutagenesis assay (Ames test), the sister chromatid exchange assay, and traditional laboratory animal toxicity tests.

Carcinogenesis: The process by which a change to a cell occurs that leads to cancer.

Cell Culture: Growth in the laboratory of cells isolated from multicellular organisms. Each culture is usually of one type. Cell culture may provide a promising alternative to animal experimentation, for example, in the testing of mutagenicity, and may also become a useful adjunct in repeated-dose toxicity testing.

Computer Simulations: The use of specially devised computer programs to simulate cells, tissues, fluids, organs, and organ systems for research purposes: to develop mathematical models and algorithms for use in toxicity testing, and to simulate experiments traditionally done with animals for educational purposes.

Distress: Usually the production of pain, anxiety, or fear. However, distress can also occur in the absence of pain. For example, an animal struggling in a restraint device may be free from pain, but may be in distress. Distress can be eased with tranquilizers.

Education: The aspect of education dealt with in this assessment is the use of animals and alternatives in the teaching of life sciences to health professionals and preprofessionals, and research scientists.

Ex vivo: Outside the living body: denoting removal of an organ, tissue or cells.

Guidelines for Animal Care and Use: Various organizations outside the federal government have adopted their own guidelines -- e.g., the American Psychological Association's *Guidelines for Ethical Conduct in the Care and Use of Animals*, which is comprehensive and has been endorsed by FASEB; the American Physiological Society's *Guiding Principles in the Care and Use of Animals*; and the American Veterinary Medical Association's *Animal Welfare Guiding Principles*. For federal guidelines, see Interagency Research Animal Committee, NRC *Guide for the Care and Use of Laboratory Animals*, and PHS *Policy*.

Institute of Laboratory Animal Research (ILAR): A component of the National Research Council, ILAR performs periodic surveys on the use of laboratory animals.

Institutional Animal Care and Use Committee (IACUC): An institutional committee that reviews research proposals and oversees housing and routine care of animals. The committee's membership generally includes the institution's attending veterinarian, a representative of the institution's administration, users of research animals, and one or more nonscientist and lay member.

Invertebrate: Any nonplant organism without a spinal column, e.g., worms, insects, and crustaceans. Invertebrates account for 90 percent of the earth's nonplant species. For the purposes of this assessment, invertebrates are not considered to be animals.

In vitro: Literally, in glass; pertaining to a biological process or reaction taking place in an artificial environment, usually a laboratory. Human and animal cells, tissues, and organs can be cultured *in vitro*. *In vitro* testing may hold some promising alternatives to animal testing, e.g., in testing for eye irritation and mutagenicity.

In vivo: Literally, in the living; pertaining to a biological process or reaction taking place in a living cell or organism.

Macrophage: A white blood cell that is very active in inflammatory responses and in engulfing foreign objects such as bacteria.

National Research Council's *Guide for the Care and Use of Laboratory Animals*: Revised in 1996, the *Guide* details standards for animal care, maintenance, and housing. It is used by many animal research facilities, both within and outside the federal government. AAALAC and PHS also

use it when assessing research facilities for accreditation.

Organ Culture: The attempt to isolate and maintain animal or human organs in *in vitro* culture. Long-term culture of whole organs is not generally feasible, but they can be sustained in cultures for short periods (hours or days).

Pain: Discomfort resulting from injury or disease. Pain can also be psychosomatic, the product of emotional stress. Pain can be induced by mechanical, thermal, electrical, or chemical stimuli, and it can be relieved by analgesics or anesthetics.

Polymerase Chain Reaction: A molecular biological system in which pieces of genetic material can be synthesized in large amounts *in vitro*. This material can be used in diagnostic testing, genetic studies, or for a large number of molecular biological purposes.

Protocol: The written plan of a scientific experiment or treatment.

Public Health Service *Policy on Humane Care and Use of Laboratory Animals*: Revised in 1985, the Policy applies to PHS-supported activities involving animals (including those of NIH). It relied on the NIH *Guide for the Care and Use of Laboratory Animals* (1985), and uses institutional committees for the assessment of programs and maintenance of records.

Reduction: Considered an alternative to animal use when fewer animals are used in research and education through changed practices, sharing of animals, or better design of experimental protocols.

Refinement: An alternative to animal use by better use and modification of existing procedures so that animals are subject to less pain and distress. Examples of such refinements are the administration of anesthetics and tranquilizers, humane destruction, and the use of noninvasive imaging techniques.

Replacement: An alternative to animal use, replacing methods using animals with those that do not. Examples include the use of a placenta instead of a whole animal for microsurgical training, the use of cell cultures instead of mice and rats, the use of non-living systems, and the use of computer programs.

Research Facility: Under the Animal Welfare Act, any individual, institution, organization, or postsecondary school that uses or intends to use live animals in research, tests, or experiments. Facilities that receive no federal support for experimental work and that either purchase animals only within their own state or that maintain their own breeding colonies are not considered research facilities under the act.

Testing: Standardized procedures that have been demonstrated to predict certain health effects in humans and animals. Testing involves the frequent repetition of well-defined procedures with measurement of standardized biological endpoints. A given test may be used to evaluate many different substances and use many animals. Testing is used to establish the efficacy, safety, and toxicity of substances and procedures.

Tissue Culture: The maintenance *in vitro* of isolated pieces of a living organism. The various cell types are still arranged as they were in the original organism and their differential functions are intact.

Toxicity Testing: The testing of substances for toxicity in order to establish conditions for their safe use. There are now more than 50,000 chemicals on the market and 500 to 1,000 new ones are introduced each year.

Vesicant: A chemical agent that causes burns and tissue destruction both internally and externally.

Veterinary Medicine: The science and art of prevention, cure and/or alleviation of disease and injury in animals. Veterinary medicine includes the management of animal care and use programs.

SECTION VIII
REFERENCES

In order of citation:

Department of Defense Directive 3216.1, "The Use of Laboratory Animals in DoD Programs," February 1, 1982; Revised, April 1995

Department of Defense Policy Memorandum, "Policy for Compliance with Federal Regulations and DoD Directives for the Care and Use of Laboratory Animals in DoD-Sponsored Programs," April 1995

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

National Academy of Sciences, National Research Council, Institute of Laboratory Animal Resources, *Guide for the Care and Use of Laboratory Animals*, 7th rev. edition, 1996

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

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

National Defense Authorization Act for Fiscal Year 1995, Report of the House Armed Services Committee, H.R. 4301, Report 103-499, May 10, 1994

Public Health Service Policy on Humane Care and Use of Laboratory Animals

Health Research Extension Act of 1985 (Public Law 99-158, November 20, 1995, "Animals in Research")

H.R. 96-1317, Department of Defense Appropriation Bill, 1981; Representative Addabbo, House Committee on Appropriations; 96th Congress, 2nd Session September 11, 1980

H.R. 97-332, Department of Defense Appropriation Bill, 1985; House Committee on Appropriation; 99th Congress, 1st Session October 24, 1985

Joint Regulation (Army Regulation 70-18; Secretary of the Navy Instruction 3900.38B; Air Force Regulation 169-2; Defense Advanced Research Projects Agency Instruction 18; Defense Nuclear Agency Instruction 3216.1B; Uniformed Services University of the Health Sciences Instruction 3203), "The Use of Animals in DoD Programs," June 1, 1984

Report to the Committees on Armed Services of the Senate and House of Representatives on Department of Defense Animal Cost and Use Programs 1993

Report to the Senate Armed Services Committee and the House of Representatives National Security Committee on Department of Defense Animal Care and Use Programs 1994, 1995 and 1996

Russell, W.M.S. and Burch, R.L., The Principles of Humane Experimental Technique, Charles C. Thomas Publishers, Springfield, IL, 1959

Army Science and Technology Master Plan, Fiscal Year 1997. Department of Army, March 1997

Title 9, Code of Federal Regulations, Animals and Animal Products, Chapter 1: "Animal and Plant Health Inspection Service", Subchapter A: "Animal Welfare"; Source: 54 FR 36147, August 31, 1989

Title 32, U.S. Code of Federal Regulations Section 219, Protection of Human Subjects in DoD-Sponsored Research

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.

John M. Deutch
Deputy Secretary of Defense

Enclosures - 3
1. References
2. Definitions
3. Guidance Documents

(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 de signs 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

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

Joseph V. Osterman

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.

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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 semiannual 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 -

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 -

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

Building _____

==========

ROOM_____ Animal Holding Area Lab Other

==========

ROOM_____ Animal Holding Area Lab Other

==========

ROOM_____ Animal Holding Area Lab Other

==========

ROOM_____ Animal Holding Area Lab Other

==========

GENERAL COMMENTS:

-OPTIONAL-
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. This form or one developed by your organization must be used to document that there were/were not minority opinions and that a majority of the IACUC members reviewed and signed the semiannual program review and facility inspection.

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

SEMIANNUAL IACUC INSPECTION/PROGRAM REVIEW SIGNATURE SHEET

The Animal Welfare Act requires IACUCs to review and inspect laboratory animal care and use programs on a semiannual basis. This form facilitates compliance with the requirement that at least a majority of members of the IACUC sign the semiannual report, and have a opportunity to express a minority opinion to the report. Minority opinions should be appended to the report in writing.

IACUC MEMBER	SIGNATURE	DATE	MINORITY OPINION YES	MINORITY OPINION NO

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 studies. 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

I. MEDICAL

Military Dentistry

Development of monoclonal antibodies against isolated of an oral bacteria implicated in the etiology of peridontal disease
Development of test kits to detect the presence of specific deleterious oral microorganisms and diagnose oral soft tissue disease

Infectious Disease

Participation in Phase 2, 3, 4 drug evaluations
Establishment of colonies for laboratory models of malaria
Identification of Oropouche, dengue, Mayaro, Venezuelan equine encephalitis, and yellow fever
Maintain worldwide resources and reference laboratories
Development of test kits for specific organisms allowing for rapid action/treatment
Development of assay for the diagnosis of hantavirus IgM and IgG
Identification of a set of 11 unique genetic sequences associated with the attenuated dengue virus
Identification of potential vectors of Venezuelan equine encephalitis, Rift Valley fever, Crimean-Congo hemorrhagic fever, and eastern equine encephalitis
Identification of an antiviral drug as a potential therapy for filoviruses such as Ebola
Development of vaccines to protect from arboviral disease
Development of a mouse malaria model to screen candidate drugs for efficacy
Prevention and diagnosis of human schistosomiasis
Evaluation of malaria candidate vaccines
Development of a vaccine against Brucellosis commonly called undulant fever
Identification and control of insect vectors of infectious disease

Chemical Defense

Management and cleanup of military nerve agents by-products
Identification of treatment compounds against lung injury by sulfur mustard gas
Identification of 33 candidate reactive components for a reactive topical skin protectant
Determining that Exogenans Buche is an effective pretreatment for organic phosphate poisoning
Development of a vaccine against ricin intoxication

Biological Defense

Successful identification of anthrax, plague, and other biological threat agents
Identification of three drugs as candidates for smallpox therapy
Demonstrated the efficacy of the recombinant F1 capsule, the recombinant V antigen, and the recombinant FI-V antigen fusion protein I protecting against pneumonic plague in rodents
Development and testing of a dengue virus vaccine
Evaluation of polyclonal antibodies against tularemia, plague, and ricin toxin
Identification of medical countermeasures for botulinum toxin
Genetic engineering of vaccine candidates
Participation in Phases 2, 3, and 4 drug evaluations

Production of polyclonal antibodies for use in the Biological Integrated Detector System
Protection of mice against lethal doses of cholera toxin

Human Systems Technology

Understanding of the circadian systems
Understanding of decompression and predict susceptibility to oxygen seizures
Detection of visual impairments caused by high G loads
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
Providing information involving environmental effects of military activities
Identification of pharmacological agents to improve performance during rapid deployment
Understanding the effects of environmental and operational stress
Development of antioxidant therapy to contribute to protection and treatment from blast exposure
Identification of useful tools to screen agents that may potentially protect from or treat post-traumatic stress disorder

Combat Casualty Care

Development of an improved topical agent for treatment of burn wounds
Development of new techniques for mechanically assisting respiration
Understanding of acute hemorrhage management related to oxygen administration to maintain blood pressure
Understanding of the mechanics of trauma and thermal injuries with complications
Development and testing of a unique bandage utilizing fibrin glue for dramatic control of massive hemorrhage
Testing for the development of lyophilized platelets suitable for transfusion in combat casualties and other trauma patients for the control of acute hemorrhage
Development of a means to store platelets in a dried state which allows years of storage with retention of platelet hemostatic ability, and agglutinating and adhesive properties
Development of a model using temporary arterial shunts to maintain limb perfusion after arterial injury
Development of a standard treatment for Brown Recluse spider bites
Development of a laser-based system to provide rapid wound stabilization at the site of injury
Development of novel therapeutic strategies for the treatment of combat-related head wounds and central nervous system (CNS) injury
Identification of useful and novel drugs for treatment of brain injury without CNS side effects
Development of therapeutic approaches to preserve oxygen delivery to the brain during post-traumatic hemorrhagic shock

Ionizing Radiation

Understanding of the bioeffects of ionizing radiation
Development of dose reconstruction for exposed individuals using chromosome painting

Other Medical Research

Identification of genetic markers and development of preventive and treatment modalities for breast cancer
Development of animal models of spatial learning and memory to test a novel treatment for learning and memory enhancement

Development of semiconductor diode laser technologies for medical field applications
Determination of the health effects of embedded depleted uranium
Performing ecological risk assessments

II. CLINICAL INVESTIGATIONS

Clinical Medicine

Development of models of acute lung injury that mimic neonatal and adult disorders
Determining that lavage surfactant administration is effective in treating non-homogeneous lung injury
Development of a mechanism by which vasopressin improves blood gases in hypoxia-induced pulmonary hypertension
Demonstration of vestibular recovery in gentamicin-exposed guinea pigs
Determination of the use of platelet gel may reduce the duration of pulmonary air leakage
Determination of the effect of selectin inhibitors for treatment or prevention of post-traumatic organ ischemia-reperfusion injury
Development of drugs that can enhance the acquisition of information and prolong the retention of memory
Determining how to prevent restenosis and promote healing
Evaluation of the use of metallic stents and balloon angioplasty

Clinical Surgery

Development of unique bony prosthetic devices and techniques to control hemorrhage and improve orthopedic repair

III. NON-MEDICAL

Assessment of the toxicity risks of environmental compounds released
Determination of the impact of munitions compounds and breakdown products on the environment
Training, care and use of marine mammals to provide economical means of underwater surveillance, object detection and marking
Antibody production and testing for use in the military's Biological Defense Program
Development of COMBAT EDGE, a +Gz-protective pressure breathing system
Development of an Advanced Technology Anti-G Suit
Development of nonlethal alternatives for compelling or deterring adversaries by using lasers
Understanding how fear and anxiety are generated and inhibited in the brain
Determination of the effects of exposure to military jet fuel

IV. TRAINING AND INSTRUCTIONAL

Graduate level medical training in surgical techniques, cardiovascular surgery, practical microsurgery, emergency surgery, obstetrical surgery dentistry, vascular and microvascular surgery pediatrics, internal medicine, and anesthesia management
Surgical laser laparoscopy training
Advanced endoscopic training that enables surgeons to become proficient in laparoscopic procedures, resulting in decreased postoperative pain, hospital stays, and recovery time
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
Pediatric bronchoesophagology training
Gynecology and obstetrics training
Neonatal resuscitation training
Battlefield anesthesia machine training
Ultrasound for surgeons training

V. ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES

Production, review and evaluation of protocols to teach personnel safe and appropriate handling techniques for care, restraint, manipulation, and sampling of animals

Development of pharmacologically based pharmacokinetic computer modeling, which reduces the number of animals required for acquiring toxicology data

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' Occupations/Avocations

Appendix H

Nonaffiliated IACUC Members' Occupations/Avocations

Occupation/Avocation	No. of Times Represented
Accountant	1
Administrator	1
Animal Science Engineer	1
Animal Welfare Informational Officer	2
Attorney	2
Biologist	5
Biomedical Researcher	1
Biostatistician	1
Chaplain/Clergy	4
Chemist	1
Church Newsletter Editor	1
Dance/Exercise Instructor	1
Exceptional Family Member Coordinator	1
Game Warden	2
Logistician	1
Occupational Health Nurse	1
Personnel Manager	2
Postal Official	1
Public Health Specialist	1
Red Cross Volunteer	1
Research Administrator	1
Safety Engineer	1
Salesman	2
Secretary/Administrative Assistant	4
Teacher	2
Veterinarian	5
Other	2

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 1997 WRAIR DoD Laboratory Animal Workshop Schedule

Appendix J

The 1997 WRAIR DoD Laboratory Animal Workshop Schedule

COURSE SUBJECT	DATES
Nonhuman Primates & Safety Badge Class 0830-1230	27 March 1997 8 May 1997 10 July 1997 8 August 1997 31 October 1997 16 January 1998
Rodents (Rats, Mice, Guinea Pigs) 0830-1300	14 March 1997 16 May 1997 6 June 1997 11 July 1997 19 September 1997 6 November 1997 23 January 1998
Lagomorphs 0830-1200	21 March 1997 9 May 1997 12 June 1997 18 July 1997 2 October 1997 5 December 1997
Ovine 0800-1300	23 May 1997 17 October 1997
Swine 0800-1300	4 April 1997 13 June 1997 21 August 1997 14 November 1997
Issues in Laboratory Animal Care and Use 0830-1130	2 May 1997 6 August 1997 24 October 1997 30 January 1998
Writing an Animal Use Protocol Using the DoD Template 1200-1330	2 May 1997 6 August 1997 24 October 1997 30 January 1998

Aseptic Techniques for Rodent Procedures 0830-1300	18 April 1997 27 June 1997 12 September 1997 11 December 1997
Operating Room Sterile Techniques 0830-1230	25 April 1997 26 September 1997 4 December 1997

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	7 July 1997
1230-1600	8 July 1997

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)	- 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)	- 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	- Attending veterinarian, facility manager, IACUC members
4. Basic Husbandry and Techniques of Laboratory Animals	- LATA video tapes and script - ACLAM slide sets with audio cassettes - USAMRIID slide set
5. Documentation of Training	- 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

American Journal of Cardiology
American Journal of Medical Sciences
American Journal of Pathology
American Journal of Physiology
American Journal of Respiratory Cell and Molecular Biology
American Journal of Tropical Medicine and Hygiene
American Journal of Respiratory Critical Care Medicine
American Sociological Review
American Surgeon
Analytical Biochemistry
Anesthesia and Analgesia
Anesthesiology
Annals of Clinical and Laboratory Science
Annals of the New York Academy of Sciences
Annals of Tropical Medicine and Parasitology
Anti-Cancer Drugs
Aquatic Mammals
Archives of Otolaryngology Head and Neck Surgery
Archives of Surgery
Archives of Toxicology
Archives of Virology
Artificial Cells Blood Substitutes and Immobilization Biotechnology
Aviation, Space and Environmental Medicine
Bioacoustics
Biochemical Journal (London)
Biochemical Pharmacology
Biochemistry
Biochemical and Biophysical Research Communications
Brain Research Bulletin
Brain Research
Cancer Research
Cell Biology and Toxicology
Cell Growth & Differentiation
Circulation
Circulation Research
Clinical and Diagnostic Laboratory Immunology
Clinical Immunology & Immunopathology
Clinical Laboratory Science
Clinical Pharmacology and Therapeutics
Critical Care Medicine
Drug and Chemical Toxicology
Drug Metabolism and Disposition
European Journal of Pharmacology

Experimental Lung Research
Environmental Toxicological Chemistry
FASEB Journal
Fems Microbiology Letters
Free Radical Research
Fundamental and Applied Toxicology
Health Physics
Hippocampus
Human and Experimental Toxicology
Human Gene Therapy
Imunology & Cell Biology
Immunology Letters
Indian Journal of Medical Research
Infection and Immunity
Inflammation
Inhalation Toxicology
International Archives of Occupational and Environmental Health
International Journal of Radiation Biology
International Journal of Toxicology
In Vitro Toxicology
Journal of American Mosquito Control Association
Journal of Analytical Toxicology
Journal of Applied Physiology
Journal of Applied Toxicology
Journal of Autonomic Pharmacology
Journal of Biological Chemistry
Journal of Biological Rhythms
Journal of Burn Care Rehabilitation
Journal of Cellular Biochemistry
Journal of Cellular Physiology
Journal of Chemistry and Physics
Journal of Chromatography-Biomedical Applications
Journal of Clinical Microbiology
Journal of Comparative Neurology
Journal of Dental Research
Journal of Experimental Medicine
Journal of Histotechnology
Journal of Immunology
Journal of Immunoassay
Journal of Industrial Microbiology
Journal of Infectious Diseases
Journal of Interferon and Cytokine Research
Journal of Investigative Medicine
Journal of Laboratory Clinical Medicine
Journal of Leukocyte Biology
Journal of Medical Entomology
Journal of Medical Primatology
Journal of Medical Virology
Journal of Neurochemistry

Journal of Nuclear Medicine
Journal of Pediatrics
Journal of Pharmacological Experimental Therapy
Journal of Pharmaceutical Science
Journal of Radiation Research
Journal of the Acoustic Society of America
Journal of the American Mosquito Control Association
Journal of the American Academy of Dermatology
Journal of the American Society for Microbiology
Journal of the Egyptian Society of Parasitology
Journal of the Undersea & Hyperbaric Medical Society
Journal of Thermal Biology
Journal of Toxicology- Cutaneous & Ocular Toxicology
Journal of Trauma
Journal of Urology
Journal of Veterinary Dentistry
Journal of Virology
Laboratory Animal Science
Lancet
Laser and Light in Ophthalmology
Life Sciences
Mammalian Genome
Marine Mammal Science
Microcirculation
Microscopy Research and Technique
Molecular Brain Research
Molecular and Chemical Neuropathology
Molecular Microbiology
Nature Genetics
Nature Medicine
Neuropharmacology
NeuroReport
Neuroscience
New England Journal of Medicine
Oncogene
Pediatric Research
Pediatric Pulmonology
Photochemistry and Photobiology
Pharmacology, Biochemistry and Behavior
Pharmacology Communications
Physiology and Behavior
Proceedings of the 1996 Medical Defense Bioscience Review
Proceedings of the National Academy of Sciences (USA)
Protein Expression and Purification
PSEBM
Psychopharmacology
Radiation Research
The Journal of Retinal and Vitreous Diseases
SEACAM

Shock
Surgical Clinics of North America
Techniques in Aquatic Toxicology
Toxicologic Pathology
Toxicologist
Toxicology and Applied Pharmacology
Toxicology and Industrial Health
Toxicology Methods
Toxicon
Transplantation Proceedings
Vaccine
Vaccine Research
Veterinary Pathology
Virology
Virus Research

Appendix M

Status of AAALAC Accreditation of DoD Animal Care and Use 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 Center for Environmental Health Research, 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
- 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
- Space and Naval Warfare Systems 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
- medical pretreatment for cyanide
- prophylactic therapeutics for chemical agents
- reactive topical skin protectant
- medical countermeasures for respiratory agents
- chemical casualty management strategies and treatments

M4: Medical Biological Defense

Includes studies in the development of medical countermeasures for:

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

M5: Human Systems Technology

Includes studies on:

- bioeffects of lasers
- laser impacts on performance
- treatment of laser-induced injury
- development of predictive models for a non-auditory exposure standard for blast overpressure

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

M6: Combat Casualty Care

Includes studies in:

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

M7: Ionizing Radiation

Includes studies on:

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

M8: Other Medical RDT&E

Includes studies in the areas of:

- breast cancer research
- pathophysiology
- neurofibromatosis research
- Gulf War illnesses
- laser research
- toxicology
- zoonosis
- free electron laser
- Defense Women's Health Research
- occupational medicine
- osteoporosis
- vectorborne diseases

NON-MEDICAL (N)

N1: Physical Protection

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

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

N2: Physical Detection

Includes studies in the development of:

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

N3: Offensive Weapons Testing

No studies were performed in this category in FY97.

N4: Other Non-Medical RDT&E

Includes studies in the areas of:

- toxicology
- marine biology
- biorobotics
- biosonar
- learning and memory physiology
- chronobiology
- bioengineering
- environmental research
- biological sensors

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 patho-physiology
- effects of tumor necrosis factor on gonado-trophic 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

- skills training

TRAINING AND INSTRUCTIONAL (T):

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

Types of training include:

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

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

T2: Other Training/Instruction

No studies were performed in this category in FY97.

ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES (A):

A1: Adjuncts to Animal Use Research

Addresses those studies and uses that 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 involving 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

No studies were performed in this category 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

(M1) Military Dentistry — Total: 267

Species	Animals Used
Mouse	230
Rabbit	9
Rat	28

(M2) Infectious Diseases — Total: 87,043

Species	Animals Used
Burro	2
Chicken	593
Cow/Bull	5
Dog	26
Gerbil	38
Goat	31
Goose	13
Guinea Pig	1,082
Hamster	2,116
Iguana	12
Jird	24
Marine Mollusc	168
Mouse	80,087
Nonhuman Primate	723
Pig/Swine	158
Pigeon	12
Rabbit	133
Rat	1,764
Sheep	56

(M3) Medical Chemical Defense — Total: 40,966

Species	Animals Used
Frog	77
Guinea Pig	3,616
Mouse	34,363
Nonhuman Primate	35
Pig/Swine	137
Rabbit	311
Rat	2,427

(M4) Medical Biological Defense **Total: 33,631**

SPECIES	ANIMALS USED
Chicken	78
Crane	16
Guinea Pig	1,850
Hamster	156
Horse	4
Mouse	30,545
Nonhuman Primate	246
Rabbit	230
Rat	506

(M5) Human Systems Technology **Total: 7,503**

SPECIES	ANIMALS USED
Dog	8
Fish	1,092
Frog	138
Guinea Pig	60
Hamster	120
Mouse	1,571
Nonhuman Primate	72
Pig/Swine	157
Rabbit	191
Rat	3,923
Sheep	171

(M6) Combat Casualty Care **Total: 11,309**

SPECIES	ANIMALS USED
Dog	16
Goat	39
Guinea Pig	210
Mouse	3,794
Nonhuman Primates	71
Pig/Swine	504
Rabbit	498
Rat	6,016
Sheep	31
Toad	130

(M7) Ionizing Radiation **Total: 6,944**

Species	Animals Used
Dog	20
Guinea Pig	59
Mouse	5,809
Rat	1,056

(M8) Other Medical RDT&E **Total: 58,700**

Species	Animals Used
Cat	6
Chicken	48
Chinchilla	48
Chipmunk	18
Dog	48
Frog	220
Goat	3
Guinea Pig	81
Hamster	6
Mouse	47,109
Nonhuman Primate	37
Opossum	6
Pig/Swine	4
Pigeon	24
Rabbit	184
Raccoon	14
Rat	7,971
Salamander	1,204
Sheep	24
Shorttail Shrew	8
Squirrel	7
Toad	311
Vole	1,319

(N1) Physical Protection **Total: 1,293**

SPECIES	ANIMALS USED
Hamster	3
Mouse	264
Nonhuman Primate	168
Pig/Swine	7
Rabbit	15
Rat	836

(N2) Physical Detection **Total: 632**

SPECIES	ANIMALS USED
Big Brown Bat	23
Chicken	20
Dog	9
Elephant Seal	20
Ferret	11
Goat	18
Mouse	295
Nonhuman Primate	5
Rabbit	131
Rat	70
Snake	30

(N3) Offensive Weapons Testing **Total: 0**

(N4) Other Non-Medical RDT&E **Total: 14,856**

SPECIES	ANIMALS USED
Bat	3
Beluga Whale	2
Bottlenose Dolphin	25
Boxfish	7
California Sea Lion	4
Cat	18
Degu	34
Dog	32
Eel	27
False Killer Whale	2
Fish	1,720
Gerbil	35
Gray Whale	1
Guinea Pig	264
Hamster	1,551
Iguana	30
Killer Whale	1
Kniff-Fish	9
Milkfish	7
Mouse	3,078
Nonhuman Primate	8
North Elephant Seal	46
Pacific Harbor Seal	1
Pacific White-Side Dolphin	3
Pig/Swine	16
Rabbit	220
Rat	5,604
Risso's Dolphin	1
Sea Turtle	2
Snake	49
Squirrel	29
Triggerfish	27
Zebra Fish	2,000

(C1) Clinical Medicine **Total: 29,663**

SPECIES	ANIMALS USED
Chinchilla	145
Dog	20
Ferret	51
Fish	140
Goat	6
Guinea Pig	188
Lamb	48
Mouse	19,388
Nonhuman Primate	48
Pig/Swine	220
Rabbit	361
Rat	8,785
Tadpole	212
Vole	51

(C2) Clinical Surgery **Total: 1,945**

SPECIES	ANIMALS USED
Dog	106
Goat	34
Mouse	386
Pig/Swine	266
Rabbit	196
Rat	929
Sheep	28

(C3) Other Clinical Investigations **Total: 404**

SPECIES	ANIMALS USED
Cat	18
Pig/Swine	162
Rabbit	44
Rat	180

(T1) Training, Education, and/or Instruction **Total: 3,702**

Species	Animals Used
Calf	3
Cat	15
Chicken	38
Dog	45
Ferret	120
Frog	62
Gerbil	4
Goat	654
Guinea Pig	91
Hamster	8
Mouse	199
Nonhuman Primate	37
Pig/Swine	618
Rabbit	137
Rat	1,646
Sheep	12
Snake	13

(T2) Other Training/Instructional **Total: 0**

(A1) Adjuncts to Animal Use Research **Total: 147**

Species	Animals Used
Beluga Whale	3
Mouse	128
Nonhuman Primate	16

(A2) Alternatives to Animal Investigation **Total: 11,939**

Species	Animals Used
African Clawed Frog	163
Bluegill	1,250
Bottlenose Dolphin	26
Guinea Pig	100
Japanese Medaka	10,400

(A3) Other Alternatives to Animal Investigation **Total: 0**

(S) Classified Secret or Above **Total: 629**

SPECIES	ANIMALS USED
Goat	54
Nonhuman Primate	44
Pig/Swine	25
Rat	506

(B) Breeding Stock **Total: 60**

SPECIES	ANIMALS USED
Nonhuman Primate	13
Rabbit	37
Rat	10

(O) Other Animal Use Purposes **Total: 4,415**

SPECIES	ANIMALS USED
Bluegill	1,600
Dog	12
Fathead Minnow	1,680
Ferret	29
Goose	5
Guinea Pig	1
Mouse	57
Nonhuman Primate	4
Rabbit	12
Rainbow Trout	1,000
Rat	15

GRAND TOTAL ANIMAL USE/RESEARCH	**316,048**

Appendix P

Alternatives

Appendix P

Alternatives

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.

- 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.

- Free-ranging honeybee (*Apis mellifera*) is used in place of animal sentinels to monitor contaminated environments.

- Use of mice replaces nonhuman primate subjects employed in biological defense studies.

- The South African clawed frog (*Xenopus laevis*) embryo replaces laboratory mammals commonly used in teratogenesis assays.

- The Japanese medaka fish replaces mice used in megamouse research on low-level exposures to carcinogens.

- The use of guinea pigs to study toxicity, stability, and potency of a new human anthrax vaccine reduces the number of rabbits and nonhuman primates (NHPs) that would have to be used to investigate the same parameters.

- Mice are used to determine dose schedule, route of immunization, and other parameters with various *Yersinia pestis* immunogens to minimize the use of NHPs.

Biochemical/ Physical Methods

- Well-tolerated, exteriorized arterial catheters are used to measure blood pressure in conscious ferrets. This increases the quality of experimental blood pressure measurements and reduces stress.

- Molecular biology techniques used to assess vasopressin receptor subtypes replaces the need to do all characterization of hormone receptor subclassifications with pharmacological tools in the whole animal.

Computer Simulations

- Rat toxicology screen has been replaced with a computer model.
- A computer model of biosonar (SCAT model) has replaced the use of 113 bats.
- Computerized patient simulator replaced goat in the nurse anesthetist training program.
- Models are used to develop hand-eye coordination before using animals to further enhance medical skills.

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.
- Pigs have replaced dogs as the model for general surgery, emergency skills, and Dental Readiness training.
- The rabbit model has replaced dogs in most platelet studies.
- Pigs have replaced dogs in the study of efficacy of arterial shunts.

Replacement Using In Vitro Cell Cultures

- Retrieval of water channels by endocytosis in renal epithelial research used in vitro techniques as well as a non-mammalian species to replace mammals.
- Primary cultured rodent hepatocytes are used to measure cytotoxic endpoints and replace in vivo testing.
- Purifying antibodies from the egg yolk of hyperimmunized chickens is a replacement to antibodies raised in serum of rabbits.
- Cell culture studies were used to identify drug action mechanisms and screen candidate compounds as an adjunct to mouse testing.
- Primary cultured rodent hepatocytes are used to measure cytotoxic endpoints and replace in vivo testing.

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

Utilization of Alternative Biological Testing Methods

- Western blot analysis of nitric oxide synthase (NOS) isoforms and polymerase chain reaction (PCR) analysis of NOS mRNA allow for replacement of some studies utilizing pharmacologic tools in the whole animal.

- Susceptible cell culture lines are determined and subsequently used for virus propagation after initial isolation in mice or hamsters. This will reduce the needed number of mice and hamsters.

- Molecular biological techniques to assess endothelins and receptor subtypes replace the need to do all characterization of hormone and receptor subclassifications with pharmacologic tools in the whole animal.

- Used PCR methods to obtain cDNA, decreasing the number of cells needed to produce a cDNA library and requiring fewer animals.

- Use of improved techniques allow detection of cytotoxic endpoints in cultured cells replacing whole live animal exposure.

- A bioreactor is used for antibody production reducing and replacing the numbers of mice.

- Reproductive toxicity screen is conducted under Screening Information Data Set Program and uses 30% less animals than EPA Health Effects Testing Guidelines.

- A nuclear magnetic resonance (NMR) technique is used to study in vivo metabolic changes on one animal over long periods of time, eliminating the need to sacrifice several animals at each time point. This approach reduces the overall number of animals required per experiment.

- The staircase up-down method to determine the lethal aerosol dose-50 for botulinum toxins B, G represents a major reduction technique in the use of animals and reduces the total number of monkeys required.

- Utilization of gnotobiotic mice will increase the experimental "effect/response" and strengthen the interpretation of experiment results.

Substitution of Computer Simulations or Other Technologies

- Non-animal training aids reduce the number of animals required.

- Multiple physiological assessments in the same animal allow for reduction in the total number of animals.

- Use of video, inanimate surgical trainer, and suture trainer decreases the number of rats required.

- Surgeon skills enhanced prior to actual procedure reduces mortality and the number of animals required.

Substitution of Another Animal Species or the Use of Humans

- The use of mice to study Ebola virus pathogenesis reduces the number of NHPs needed to gain a full understanding of ebola infection in man.

- The use of human volunteers with brain damage has reduced the need to perform lesion and recording experiments on non-human primates, thus fewer animals have been used.

- The use of guinea pigs in anthrax vaccine research reduces the number of rabbits and NHPs used to investigate *Bacillus anthracis* strain difference.

Changes in Endpoint Measurements

- An NMR technique is used to study in vivo metabolic changes on one animal over long periods of time, eliminating the need to sacrifice several animals at each time point.

- Study endpoints are adjusted when percentage of parasitemia rises to 3%-5% and/or Hct decreases by 50% over preinfection values in malaria candidate vaccines studies, withdrawing monkeys from study.

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

- All chicks will be anesthetized for the mosquito feeding portion of the study instead of using physical restraint as in previous protocols.

- Monkeys will be anesthetized during blood collection, immunizations, and biopsy collections to decrease stress. Gastric biopsies are shared with other investigators to maximally utilize tissues.

- 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.

- Early intervention adjustment to the endpoint during toxicity testing phase will prevent unnecessary pain and suffering to symptomatic animals.

- Knowledge of the disease process in the animal model allows for sick animals to be assessed more accurately and to be euthanized prior to unnecessary pain and suffering.

- 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.

- Incorporation of long-acting postop analgesics reduces or eliminates pain.

Reduce Distress

- Research on Wound Healing Potential of Human Keratinocyte/Fibroblast constructs includes both a short-term and a long-term phase designed to prevent continuation of the study if benefits are not found during the short-term phase.

- Animals are housed in social settings (i.e., pairs or groups) in an enriched environment (e.g., nestboxes, toys).

- The use of radiotelemetry is a refinement by lowering the stress-induced artifact and by providing long, continuous periods of data collection.

- Physiological parameters are continuously monitored by telemetry in an unrestrained animal.

- Animals serve only as a source of red blood cells. Cellular pathophysiology of heat stroke is studied using an in-vitro guinea pig red blood cell system.

- Telemetry is used to collect body temperature data and decrease stress associated with manual procedures.

- The restraint technique for phlebotomy uses isoflurane gas anesthetic to reduce distress and discomfort while enabling the phlebotomist to obtain adequate blood samples quickly and effectively.

- The number of intubations per ferret per session are limited.

- Immunogens are prescreened in vitro prior to administration to test animals.

- Use of Laser-Doppler flowmetry allows continuous measurements without the removal of brain tissue. Also, multiple blood flow determinations can be made in a single rat, which can be maintained for other subsequent correlative evaluations.

Research Models and Animal Alternatives

- Initial training utilizes inanimate training aids thus providing students with a basic level of skill to enable better skills with live tissue.

- Using 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 makes bilateral femoral artery cutdown unnecessary.

- During laparoscopic training, students practice before attempting the procedures on an anesthetized animal.

Environmental Enrichment and Improved Animal Handling

- Additional social contact and sexual activity allowed and encouraged in rhesus monkey breeding colony.

- Engaging rhesus monkeys in behavioral interaction that emulates the essential features of natural foraging provides environmental enrichment for them and refines the environmental conditions of captive primates.

- Technique videos and non-animal models are used to improve technique prior to animal use.

Appendix Q

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